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THE MINIMUM EFFECTIVE DOSE OF LIDOCAINE NEEDED TO BLOCK EVOKED POTENTIALS IN THE SCIATIC NERVE OF THE RAT

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ABSTRACT

Local anesthetics are used daily by anesthesia providers to perform spinal, epidural, and peripheral nerve blocks. Large volumes of local anesthetic are used for peripheral nerve blocks especially if more than one block is performed on a patient. These local anesthetics can have side effects such as nerve compression, nerve damage, or even toxic plasma levels that can lead to seizures. Using less volumes and concentrations of these anesthetics would reduce the chances of these complications. To discover the minimum amount of local anesthetic needed intraneurally, an *in vivo* model of the rat sciatic nerve was used. In the anesthetized rat, the sciatic nerve was surgically exposed and then injected in the subperineural space with either 10 or 20 µl of 2% lidocaine or a control solution. The proximal end of the nerve (at the greater sciatic notch) was electrically stimulated and the tibial division of the nerve (near the ankle) was used for recording the compound action potential (CAP). The averaged CAPs were recorded periodically for up to an hour. The data displayed a trend of 20µl of 2% lidocaine blocking the CAPs and that 10µl of 2% lidocaine did not consistently block the CAPs. The data suggests that 20µl is the minimum dose of 2% lidocaine needed intraneurally to block evoked potentials in muscle and rapidly conducting sensory signals, including fast pain.

KEYWORD: lidocaine, rats, minimum dose, *in vivo*, sciatic nerve

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by

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DEDICATION

I dedicate this thesis to Deanna, my wife, Hannah and Jacob, my children. Their love and support have given me the ability to obtain any dream, and without them my dreams would be meaningless.

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CHAPTER ONE: INTRODUCTION

Nurses have been delivering general anesthesia since Sister Mary Bernard became the first identified nurse anesthetist in 1877 (Bankert, 1993). Regional anesthesia, anesthetizing nerves of the peripheral nervous system, currently constitutes a large portion of the nurse anesthetists' practice. The first regional block was done in 1885 by Halstead in an experiment in which he blocked the brachial plexus with cocaine (Stoelting & Miller, 1994). Today, regional blockade of most sensory peripheral nerves is done for procedures such as operations on shoulders, hands, legs, ankles, and feet. Anesthesia providers are taught to use recommended volumes and concentrations, with or without vasodilators, of local anesthetic to perform these blocks. For example, in Stoelting and Miller's book, Basics of Anesthesia, 25 ml of local anesthetic is recommended to block a sciatic nerve, 25 to 40 ml to block the brachial plexus or the axillary nerve, and 10 to 20 ml for the femoral nerve. Even though many local anesthetics are available from which the anesthesia provider may choose, there are still potential problems (Stoelting, 1991).

Statement of the Problem

Recommended volumes of anesthetic to perform a regional blockade of a peripheral nerve can cause adverse sequelae such as nerve damage from local anesthetic toxicity, tissue edema, or systemic toxicity which could lead to seizures (Stoelting, 1991: Kalichman, Powell, & Myers, 1989). A large volume of local anesthetic is used for regional anesthetic procedures to ensure that the nerve becomes anesthetized. If more than one block is performed, such as sciatic and femoral nerve blocks, the total dose of

local anesthetic that may be absorbed systemically could reach toxic levels. Also, local anesthetics have direct toxic effects on nerve tissue and may cause injury and edema.

An *in vivo* model has been reported in abstract form, by Paris, Pahno, Rigamonti, Jimmerson, & Seng in 1990, to study the minimum effective dose of a local anesthetic to block nerve conduction in the sciatic nerve of an animal. This thesis will describe what volume of commercially prepared local anesthetic, in an intraneural injection, is needed to block the sciatic nerve of the rat using the model developed by Paris et al. Neural signals, recorded and analyzed by a computer, will be used to determine the minimum dose needed to block muscle response and the fast conducting sensory system, including the fibers commonly thought to conduct "fast pain" signals.

Background

Peripheral Nervous System

The nervous system can be divided into two parts, the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS consists of the brain and the spinal cord, and although very important, it is not the target of 2% lidocaine which is studied in this thesis. Thus, this section will be limited to a brief description of the PNS.

The peripheral nervous system consists of 31 pairs of spinal nerves, 12 pairs of cranial nerves, and the autonomic nervous system (ANS). Due to the scope of this thesis, only spinal nerves, which include the sciatic nerve, will be described. Gilman and Newman (1996) describe the PNS in their book, Manter and Gatz's Essentials of Clinical Neuroanatomy and Neurophysiology. They wrote about two classes of spinal nerves,

efferent and afferent nerves. Afferent nerves are sensory nerves that have their cell bodies located in a ganglia. For example, the cell body for an afferent spinal neuron is located in the dorsal root ganglion. Sensory nerves send nerve impulses towards the spinal cord in response to stimuli such as pain, temperature, vibration, etc. These nerves may synapse in the dorsal horn of the spinal cord's gray matter. The gray matter of the spinal cord is subdivided into 10 regions termed laminae. The dorsal horn is comprised of laminae I to VI. Lamina I, also called the marginal zone, and lamina II, also called the substantia gelatinosa, receive impulses from C-fiber and A-δ fiber nerves that are stimulated by pain, temperature, touch, and pressure. Laminae III and IV, also termed the nucleus proprius, and laminae V and VI receive input from A-α and A-β fiber nerves that are stimulated by light touch, vibration, and muscle spindles as well as Golgi tendon organs which signal proprioception.

The nerve impulses are then either processed in the spinal cord or in the brain (Gilman & Newman, 1996). Efferent nerves send nerve impulses towards effector organs, muscles, glands, etc. Efferent nerves have their cell bodies located in the ventral or intermediate horns of the spinal cord's gray matter. Lamina VII contains the cell bodies of the B-fiber, autonomic nerves, and lamina IX contains the cell bodies of the A-α and A-γ fibers, motor nerves. Afferent and efferent spinal nerve fibers are bound together in an epineural sheath and innervate distal structures of the body. They are collectively called a nerve. For example, the sciatic nerve is composed of efferent and afferent nerves of all fiber classifications except B fibers. The sciatic nerve carries afferent impulses from

the skin, muscles, joints, and fascia of the leg and efferent impulses to the muscles, glands, and smooth muscle of the leg.

Nerve Conduction

Strichartz (1988) described nerve conduction in the book, Neural Blockade in Clinical Anesthesia and Management of Pain. He wrote that sensory and motor messages are transported along a nerve in the form of an electrical impulse. The sciatic nerve consists of thousands of individual axons. An electrical potential is maintained in each axon by maintaining a concentration gradient across its membrane. In the resting state, there is a slightly higher concentration of potassium (K^+) ions intracellularly and a higher concentration of sodium (Na^+) ions extracellularly. This, along with the semi-permeable cell membrane, creates a resting membrane potential of approximately –70 to -80 mV. As Na^+ ions enter the cell, the resting membrane potential becomes more positive (depolarizes). As the membranes potential depolarizes, it reaches a state called threshold at which time voltage gated Na^+ channels open up and a rapid influx of Na^+ ions occurs. This is the start of an action potential. An action potential will peak at approximately 110 mV even with different, short duration, stimuli strengths as long as the membrane threshold is reached. Voltage gated K^+ channels also open when threshold is reached but at a slower rate, so when the Na^+ channels close, the membrane potential becomes more negative (repolarizes) until it returns approximately to its resting state. During the action potential, the Na^+ ions that entered the axon then cause the distal membrane, such as the

next node of Ranvier in a myelinated fiber, to depolarize and when it reaches threshold, another action potential is generated. This is the manner in which an impulse travels down a nerve. The conduction of this impulse is aided in myelinated nerve fiber by Schwann cells. In myelinated fibers each Schwann cell is wrapped many times around the nerve axon. The gap between these protective cells is called the node of Ranvier. The voltage gated Na^+ channels that produce the action potential are located predominantly in these nodes. This allows the action potential to travel along the nerve from node to node with the benefits of faster conduction and less Na^+ crossing the membrane than an unmyelinated axon (Gilman & Newman, 1996).

During an action potential, the voltage gated Na^+ channels are in the open position at the start of the action potential and then go into an inactivated or closed state (Strichartz, 1988). These Na^+ channels must then reset to their resting state before they can be opened again. This time while the Na^+ channels are resetting is called the refractory period. The refractory period has two phases. The absolute refractory period, in which the membrane cannot be further stimulated to produce another action potential, lasts from the time the membrane reaches threshold to the time the membrane is partially repolarized. This is followed by the relative refractory period during which the membrane can respond to a strong stimulus and produce another action potential. An electrically stimulated action potential that begins at the node of Ranvier can travel in either direction up or down the axon.

Local Anesthetics

Local anesthetics were first used in 1884 when Carl Koller used cocaine topically for his ophthalmic procedures (Duncum, 1947). William Halstead performed the first regional anesthesia in 1844 by blocking the brachial plexus with cocaine. In 1885 Corning placed cocaine extradurally to anesthetize the spinal cord. August Bier, in 1899, performed the first planned spinal anesthetic. Alfred Einhorn developed procaine in 1904 to be used as a local anesthetic with less side effects than cocaine. The first of the amide local anesthetics, lidocaine, was made by Nils Lofgren in 1943.

Local anesthetics are weak bases with pKa's higher than normal physiologic pH which means that more than half of the drug will exist in the non-active ionized form in the blood (Stoelting, 1991). There are two different groups of local anesthetics, the esters (procaine, chloroprocaine, tetracaine) and the amides (lidocaine, mepivacaine, bupivacaine, etidocaine, prilocaine, ropivacaine). They are grouped by the specific type of hydrocarbon chain they have between their lipophilic and their hydrophilic ends. The ester local anesthetics are metabolized mainly by plasma cholinesterase. The amide local anesthetics are mainly metabolized in the liver by microsomal enzymes. Allergic reactions are more common with the ester local anesthetics because they are metabolized to structures that are similar to para-aminobenzoic acid. Also, preservatives in local anesthetics that resemble para-aminobenzoic acid may also cause allergic reactions. Systemic toxicity is more likely to occur with use of amide local anesthetics because they are metabolized slower.

Local anesthetics work by blocking passage of sodium ions through voltage gated sodium channels located in the cellular membrane of a nerve (Stoelting, 1991). The anesthetics attach to a specific receptor on the voltage gated sodium channels that respond to nerve impulses. Blockage of this flow of sodium ions will stop the membrane from reaching threshold and an action potential from propagating.

Classification of Nerve Fibers

Differentiating fibers of an amphibian nerve can be accomplished by recording the nerve's compound action potential (Gasser & Erlanger, 1929). Electrodes are placed on a nerve to record nerve impulses as they travel the length of the nerve fibers. Because nerve fibers have different conduction velocities, relative to their diameter and myelination properties, these impulses will reach the electrodes at different times. The larger diameter, myelinated fibers have the greatest conduction velocities (Gilman & Newman, 1996). The conduction velocities, from largest to smallest diameter in mammals, are 120 m/sec for Aα fibers, 70 m/sec for Aβ fibers, 40 m/sec for Aγ fibers, 15 m/sec for Aδ fibers, 14 m/sec for B fibers, and 2 m/sec for C fibers. An oscilloscope records these impulses and the tracing is displayed on a grid with the y-axis being amplitude and the x-axis being time. The stronger impulses will create greater voltage amplitude curves. The faster impulses will be recorded first. In a normally functioning nerve that contains these different fiber groups, the first peak will have the greatest amplitude and represent the Aα fibers. These fibers terminate in striated muscle and are responsible for muscle twitch. The next peak will be smaller and represent the Aβ fibers. This continues in

order of fastest conduction velocity to the slowest. By interpreting the oscilloscope tracing, data can be obtained on the blocking effects of local anesthetics on nerve fibers. As nerve fibers were blocked, the tracings would show lessening amplitudes for that group of fibers.

Summary

This chapter briefly discussed what potential problems there are with administering a regional anesthetic block to a peripheral nerve. It described the main components of this thesis, the PNS, which is a targeted anatomy in regional anesthesia, nerve conduction, and the basics on how impulses are transmitted by nerve fibers. Also, the other major component of this thesis, local anesthetics, was described. The mechanism of action of local anesthetics and how they block conduction of nerve impulses, which is the framework for this thesis, is described in chapter three.

CHAPTER TWO: REVIEW OF THE LITERATURE

Introduction

The literature describes how evoked potentials are blocked, how local anesthetics work, and what damage local anesthetics can create. This review forms the physiologic framework for this study. Evoked potentials are blocked at different rates in each kind of nerve fiber. This is termed differential blockade. This can usually be seen clinically when a nerve is anesthetized, the patient will lose neural function in the following order: autonomics, temperature, pain, touch, pressure, motor, vibratory, and lastly proprioception. Also, when a patient receives an epidural block, autonomic response is blocked two to four dermatomes higher and sensory is blocked two dermatomes higher than motor. It is still not fully understood how this happens, but there are different possible explanations. Local anesthetics have been well described in the literature and their toxic effects on nerve fibers have also been reported. Although Cauda Equina Syndrome and Transient Radicular Irritation are important injuries from lidocaine anesthesia, this thesis will focus on the sciatic nerve as a model which may be important to the other syndromes.

Blocking Evoked Potentials

Nobel Prize recipients Gasser and Erlanger (1929) described the relation between amphibian nerve fiber size and blockage of its nerve impulses by cocaine and compression. They found that there was a differential blockade of nerve fibers but there were no experimental data to show what quality of the nerve fiber made this happen.

They had previously found that velocity of nerve conduction related directly to the fibers diameter. This was used to determine differential blockade by oscilloscope tracings. Gasser and Erlanger found that differential blockade made by cocaine in a frog nerve was inconsistent or not even present. They found that in every mammalian nerve, that they tested, that the smaller fibers were blocked before the larger fibers. In one experiment, they found that the Aδ wave, as seen on the oscilloscope, was always completely blocked before the Aαβγ group. They did another experiment to focus on the Aαβγ group and found, "γ was more affected than β, and β more than α; but during the blocking of the slower waves the faster waves were undergoing alterations in form" (p.587-8). They concluded with the sum of all their experiments that the amphibian nerve size is a determining factor in how susceptible it is to cocaine but that during differential blockade, fibers do not drop out strictly based upon their diameter.

Jaffe and Rowe (1996) used an *in vitro* model to try and explain differential nerve block. Their model used lumbar dorsal roots and cervical area vagus nerves from adult Sprague-Dawley rats. The section of nerve was removed and placed into an artificial cerebrospinal fluid solution where the distal end was stimulated with 0.3 Hz, 0.2 ms duration pulses. The proximal end was divided into small sections of one to three fibers in which recordings were taken. After recording baseline data, the artificial cerebrospinal fluid was replaced with a solution containing 150μM of lidocaine (Astra). If there were axons that were not blocked at this concentration, they increased the lidocaine to 260μM and then 540μM. Jaffe and Rowe's found that of the dorsal root axons, 88% of

unmyelinated and 100% of myelinated were blocked with 520μM of lidocaine. Their results comparing the vagus nerve axons and dorsal root axons with 260μM lidocaine showed that fewer unmyelinated and myelinated vagal axons were blocked but that only the myelinated difference was statistically significant. They concluded that, "compared to unmyelinated axons, myelinated dorsal root axons are significantly more sensitive to steady-state sodium channel blocking effects of lidocaine" (p. 1463). They hypothesized that one reason differential block from an epidural block occurs is because with short sections of spinal roots being anesthetized, the large diameter myelinated axons will have fewer nodes exposed to the local anesthetic.

Raymond, Steffensen, Gugino, and Strichartz (1989) performed an *in vitro* study to show how exposure length helps determine nerve impulse blockade. They exposed different lengths (5 to 30mm) of the sciatic nerve of frogs to various concentrations of lidocaine (Astra). They electrically stimulated the nerve and recorded the compound action potentials from the distal end. The stimulating and recording ends were not exposed to the local anesthetic. The researchers also took recordings from single fibers that were teased from the distal end. In these experiments, the portion of nerve trunk exposed still had its sheath intact. The researchers found that the slower, small diameter, myelinated fibers were not more susceptible to being blocked by lidocaine with the lengths of exposure that they used. Their single fiber testing lead them to conclude, "at short exposure lengths, more than twice as much anesthetic was required than at the longest exposure lengths (25-35mm)" (p. 567). Finally, Raymond, et al. found, "that at

any given concentration of lidocaine there is direct relation between the incidence of block in a fiber population and the length of nerve exposed to LA [local anesthetics]" (p. 568).

Huang, Thalhammer, Raymond, and Strichartz (1997) tested different afferent rat sciatic nerve fibers *in vivo* to determine their sensitivity to lidocaine. They exposed the sciatic nerve and the tibial division, placed a 22mm section in a bathing tube in which lidocaine could be added or washed out, and then they separated out nerve fibers by A-β, A-δ, and C at the proximal end to record. The distal end of the nerve was stimulated and the bathing tube was infused with different concentrations of lidocaine. Also, the researchers used ^{14}C-lidocaine to determine concentration of the lidocaine that diffused across the neural sheath into the nerve fiber. They found that a steady state of lidocaine uptake was obtained at approximately 20 minutes and that the mean lidocaine uptake was 1.0 ± 0.2 nmol/mg of wet nerve. Their results of differential blockade showed, "when characterized by their median blocking concentrations (IC_{50}s), nociceptive Aδ-fibers were blocked at the lowest concentration, 0.32 mM, compared with 0.41 mM for LTM [light touch mechanoreceptor] Aβ-fibers and 0.80 mM for of nociceptive C-fibers" (p. 808). They concluded that diameter does not determine a nerve's susceptibility to local anesthetics.

Local Anesthetics

Butterworth and Strichartz (1990) reviewed the mechanisms of local anesthetics on voltage gated Na^+ channels. Local anesthetics have both a tonic and a phasic mode of blocking the Na^+ channel. In the tonic phase, the local anesthetic binds to the Na^+ channel

while the channel is closed resulting in less Na^+ flowing into the cell. During the phasic block, the local anesthetic binds to either the open, activated, or inactivated Na^+ channel. There are two theories for this, one of which is that the site that local anesthetics binds to is altered when the Na^+ channel changes states and this causes the local anesthetic to be bound tighter to open and inactivated states. The other hypothesis is that the receptor is guarded and can be accessed when the Na^+ channel is such that the guarding is less. The researchers did write that one property of a local anesthetic is whether it produces more tonic block or more phasic block.

Butterworth and Strichartz (1990) described the structure of a Na^+ channel as being a polypeptide, which could be divided into four sections, each containing six to eight amino acids. The four sections form four quadrants of a cylinder with the Na^+ channel being in the middle. The amino acid labeled "c" for each quadrant is located in the center and these four amino acids form the Na^+ channel. The Na^+ channel opens when the "g" amino acids move intracellularly and the "d" amino acids move extracellularly allowing the "c" amino acids to move away from each other and thus opening the Na^+ channel. The researchers wrote, "we propose that LAs [local anesthetics] bind at the dipole-containing helices such as to inhibit their arrangement in response to membrane depolarization" (p. 730).

Recently, Popitz-Bergez, Leeson, Strichartz, and Thalhammer (1995) conducted an *in vivo* experiment to determine the amount of intraneural Lidocaine that corresponds to different phases of blockade. All the animals were given a sciatic nerve block, with 0.1

ml of 1.0% ^{14}C lidocaine HCl, by a single injection between the greater trochanter and the ischial tuberosity. The animals were euthanized during either full blockade, when deep pain returned, or when complete functions returned in order to measure the amount of intraneural lidocaine. This was determined by performing an examination that had been previously described in the literature by Thalhammer. Their results showed that for total blockade, averaging 5 to 35 minutes, there was only 1.6 ± 0.12% of the total dose of lidocaine injected found intraneurally. When the response to deep pain returned, average 32 to 40 minutes, there was only 0.33 ± 0.035% of the total dose of lidocaine detected. Finally, when all functions returned, they detected only 0.065 ± 0.035% of the total dose of lidocaine injected. In their discussion, they wrote, "It is interesting that the minimal ratio of drug dose to body weight producing a full block of function seems to be the same for rats and humans" (p. 590). For this statement, the researchers compared their results with those of Cousins and Bridenbaugh who wrote that a sciatic nerve block in humans can be accomplished with 10 cc of 2% Lidocaine. They calculated the body weight to drug dose ratio was exactly the same when comparing a 70 kg human and a 350 gram rat, which is the weight of the animals that these researchers studied.

Popitz-Bergez, Leeson, Strichartz, and Thalhammer (1997) repeated this study to compare intraneural lidocaine uptake between pregnant and nonpregnant male and female rats. In comparing pregnant and nonpregnant rats, they wrote, "The block of deep pain sensation was prolonged by 45% in pregnant rats and that the amount of local anesthetic present in the nerve at the time of deep pain recovery was also significantly less in

pregnant rats than in nonpregnant rats" (p. 369). This study yielded similar results for the male rats as their previous study. When deep pain sensation returned, the intraneural lidocaine content was 0.34 ± 0.03% of the total dose injected.

Nerve Damage with Local Anesthetics

Kalichman, Moorhouse, Powell, and Myers (1993) examined the toxicity of local anesthetics to nerves. They anesthetized the exposed sciatic nerve of Sprague-Dawley rats with 1 ml of either etidocaine, lidocaine, 2-chloroprocaine, or procaine. Then, 48 hours after applying the local anesthetic, they removed the section of nerve that was exposed to the anesthetic, sectioned it one micron thick and stained the sections with p-phenylenediamine and examined them under light microscopy. These sections were given nerve injury scores of 0 (no injury) to 4 (more than half of the fibers injured in one fascicle or if there was nerve damage in three or more fascicles). The nerve sections were also assessed for edema. Kalichman et. al. also experimented with 57 other Sprague-Dawley rats to find the concentration of the four local anesthetics that would produce 50% motor nerve conduction block. This was done to be able to compare nerve injury to anesthetic potency. Anesthetic (250µl) was applied to each side of the sciatic nerve for a total of 500µl. Then they recorded EMG responses from the interosseous muscles on the ipsilateral foot with an oscilloscope. They used this measure to determine when the amplitude was decreased to 50% of baseline.

Kalichman et al. (1993) found that all four of the anesthetics produced edema. In comparing nerve injury and potency of anesthetic, they wrote, "these data support the

hypothesis that local anesthetic neural toxicity parallels potency for producing local anesthesia" (p. 239). They also found that nerve injury was more severe in small fascicles and edema was more likely to be formed in large fascicles. Lidocaine, in their study, was shown to have 88% to 100% conduction block with 1% solution and a nerve injury score of 2.2 +/- 0.6 at 1.3% solution. A nerve score of 2 was obtained if up to one forth of the fibers in a fascicle were injured.

Kalichman, et al. (1989) performed a similar study earlier in which they also looked at four different local anesthetics and their effects on nerve tissue. They wrote that, "at high concentrations, and sometimes even at clinical concentrations, local anesthetics are capable of producing dose-dependent injury to peripheral nerve" (p. 406). In this study, the researchers injected 1 ml of local anesthetic perineurally on the sciatic nerve of the rat. The local anesthetics tested were lidocaine (1.5%, 2.1%, 3%), 2-chloroprocaine (2%, 2.8%, 4%), etidocaine (1%, 1.4%, 2%), and procaine (5%, 7%, 10%). The sciatic nerve was removed 48 hours after the injection and dissected into 1μ thick sections to be studied with light microscopy. A myelinated nerve fiber was considered injured if it was swollen, had a disintegrated axon, the nerve fiber was dystrophic, or if it became thinly myelinated or demyelinated. They found that nerve injury was significantly different from the control solution only with the highest concentrations. The researchers also looked at edema in the nerve tissue. They hypothesized that at clinical concentrations, the edema most likely would not cause any lasting neurological effects. Kalichman et al. reported that it would be possible to produce lasting deficits if higher

concentrations were used, epinephrine was used, or if a dose was repeated. About nerve injury, the researchers concluded that, “four very different local anesthetic agents produce patterns of nerve injury with relative potencies quite similar to those for producing local anesthesia” (p. 410).

CHAPTER THREE: FRAMEWORK OF THE STUDY

This study will have a physiologic framework based on the current research and interpretation of how nerves transmit impulses and how local anesthetics exert their effects. The framework has been described in the introductory chapter during the background section.

Local Anesthetics

In his description of the mechanism of action for local anesthetics, Stoelting (1991) wrote that local anesthetics bind selectively to Na^+ channels when they are in an inactivated-closed state which prevents the Na^+ channels from changing to a resting or open-active state. How quickly the local anesthetic works is determined by the anesthetic's pK. The pK of an anesthetic equals the pH at which it exists 50% in the nonionized form. The more nonionized form an anesthetic is in, the quicker it will be able to work. The nonionized form is able to cross the cell membrane and then, in the lower pH of the intracellular fluid, become ionized and attach to the receptor which is located on the intracellular side of the sodium channel. The duration of the block can be increased by adding epinephrine, giving a larger dose, or by giving a more lipid soluble anesthetic.

The least concentration of a local anesthetic that is needed to block nerve impulses is termed minimum concentration (Cm). An increase in pH at the site of injection will decrease the Cm of the local anesthetic. Also, the high frequency motor fibers require less local anesthetic than lower frequency sensory fibers. Even though motor fibers have higher frequencies and therefore should be blocked easier and with less anesthetic,

clinically it is the sensory fibers that are seen being blocked first and for the longest duration. This happens because the sensory fibers have less length that must be exposed to the anesthetic for the nerve impulses to be blocked. The C fibers are unmyelinated and the A-δ fibers are lightly myelinated.

Another property of LAs is that they cause frequency-dependent blockade. The ionized, intracellular LA only enters the Na^+ channel in the activated open state, so the more the nerve transmits evoked potentials (frequency) the more the Na^+ channels will be in the activated open state which allows the LA to bind to it. LAs also cause differential conduction blockade. This is displayed by B-fibers blocking first and with lesser amounts of LA, the C-fibers and Aδ-fibers blocking next with higher concentrations of LA, and the rest of the A-fibers blocking last and requiring the highest concentration of LA.

LOCAL ANESTHETICS AND NERVE CONDUCTION

Ichiji Tasaki (1982), in his book Physiology and electrochemistry of nerve fibers, notes the effects of anesthetics on nerve impulses. He wrote, "local anesthetics prolong the least interval while they shorten the absolute refractory period" (p. 79). Also, the higher concentration of anesthetic will decrease the time needed to block conduction in the nerve. Tasaki stated that cocaine, "conduction block takes place at a concentration high enough to reduce the action potential amplitude of the node to about _ of the normal value". Another concept is that the more length of a nerve exposed to an anesthetic, the quicker nerve conduction is blocked. This occurs until a length of approximately 6mm is

exposed to a local anesthetic. In large diameter myelinated fibers, 6mm corresponds to an average of 2.5 nodes. Blockade of nerve conduction occurs more rapidly in small diameter myelinated fibers because they have more nodes in 6 mm than large diameter fibers.

CHAPTER FOUR: METHODOLOGY

This is a quantitative, descriptive study to determine the minimum amount of lidocaine (2%) needed to block recorded, averaged, evoked potentials. The model reported by Paris et al. was followed. Animal procedures for these experiments were approved by the Laboratory Animal Review Board at the Uniformed Services University of the Health Sciences. A total of 16 adult Sprague-Dawley rats were operated on, preliminary data and experimental procedures were collected in six animals and 10 animals yielded useful data. The animals were numbered sequentially 1 to 16. Five animals were placed in each experimental group, control group data were obtained from three animals before they were placed in the experimental group.

The animals were initially anesthetized using Nembutal Sodium Solution (Abbott Laboratories, North Chicago, IL) 75 mg/kg ip. Supplemental doses were administered as needed, determined by the presence of a corneal reflex, and then given every hour after administration of the muscle relaxant. The trachea was surgically exposed and a tracheal tube was inserted. The internal jugular vein was cannulated, in order to administer d-Tubocurarine, and kept patent with Heparin Sodium Injection, USP (Pharmacia and Upjohn Company, Kalamazoo, MI). The left sciatic nerve was then surgically exposed at the greater sciatic notch and the ankle with the aid of a surgical microscope (Zeiss).

A pair of “stimulating” electrodes was placed on the nerve at the greater sciatic notch and another pair of “recording” electrodes was placed on the tibial nerve near the ankle (Figure 2). The electrodes were embedded in a low melting point wax in order to

avoid possible signal interference from surrounding tissue and also to ensure that the electrical impulse would go only to the nerve. The nerve was crushed and tied off proximal to the stimulating electrodes and distal to the recording electrodes. The d-Tubocurarine Chloride (Sigma Chemical Company, St. Louis, MO) 0.04mg/kg was administered through the cannulated internal jugular vein. The animal was then artificially ventilated via a Harvard Apparatus Rodent Respirator. The respirations were 50 breaths per minute, with a tidal volume of 1 cc per 100g of rat mass. Core temperature was physiologically maintained by placing the animal on a heating pad. Rectal temperature monitored with Physitemp BAT-12 (Physitemp Instruments Inc., Clifton, NJ).

The recording electrodes were connected to the differential inputs of a DAM 50 (World Precision Instruments, Inc., Sarasota, FL) biological amplifier. Gains were between 100 and 1000. Bandwidth was 0.01 to 3 kHz. The amplifier output was displayed on an oscilloscope for continuous monitoring and recorded on a MacIntosh computer. A MacLab/8 system connected to the computer running Scope v3.5 software (Analog Digital Instruments) was used to initiate the electrical stimulus and to record the nerve impulses. The nerve was stimulated with a 0.2 ms duration pulse every 5 seconds delivered by a Grass S44 Stimulator through a Grass SIU5 Stimulus Isolation Unit (Grass Medical Instruments, Quincy, Mass). Thirty-two responses were averaged. Baseline neurograms were obtained at ten times threshold. Stimulus threshold was determined to be the minimum amount of voltage needed to create a detectable positive deflection in the recorded neurogram. After baseline values were obtained, an injection of clinical grade

lidocaine 2% (Abbott Laboratories, North Chicago, IL) or control solution was made inside the nerve sheath via a glass micropipette, pulled to a OD<30µ tip, and then post injection recordings were obtained. Recordings were made for one hour after injection.

The trials were performed at the volumes of 10µl and 20µl. The control group (consisting of 3 animals) was injected with a modified Krebs-Henselit solution ($NaHCO_3$ 27.2mM, NaCl 118mM, KCl 4.8mM, KH_2PO_4 1.0mM, $MgSO_4$ 1.2mM, $CaCl_2$ 2.5mM, and glucose 11.1mM osmolarity of 300 +/- 5 mosM and a pH of 7.4 +/- 0.05) to determine the effect volume may play in blocking evoked potentials. After compound action potentials return to baseline, one animal was then given an additional of rat Ringer solution with Methylene Blue to measure the diffusion distance of the 10µl volume in the nerve's sheath. All solutions (control or 2% lidocaine) were injected by the same investigator into the nerve under microscopic conditions, distal to the stimulating electrodes, using a glass micropipette placed under the perineurium.

The remaining animals (5 per volume group) were injected with lidocaine 2% which is sold commercially to be used clinically on humans. Data collected include: weight of rat, volume of lidocaine used, initial recordings of the compound action potentials, the amplitude, latency, area under the curve until blockage of evoked potentials was complete, and spread distance of the solution. The data collected was described in a quantitative manner. The animals were euthanized, after the experiment end point and before the anesthesia wore off, by giving 200mg/kg ip of Nembutal. The animals' chests were also opened to create a pneumothorax. After the animals were

euthanized, the distance between the stimulating and recording electrodes was measured by dissecting the sciatic nerve. This was used to calculate the conduction velocity of the nerve fibers.

CHAPTER FIVE: ANALYSIS OF THE DATA

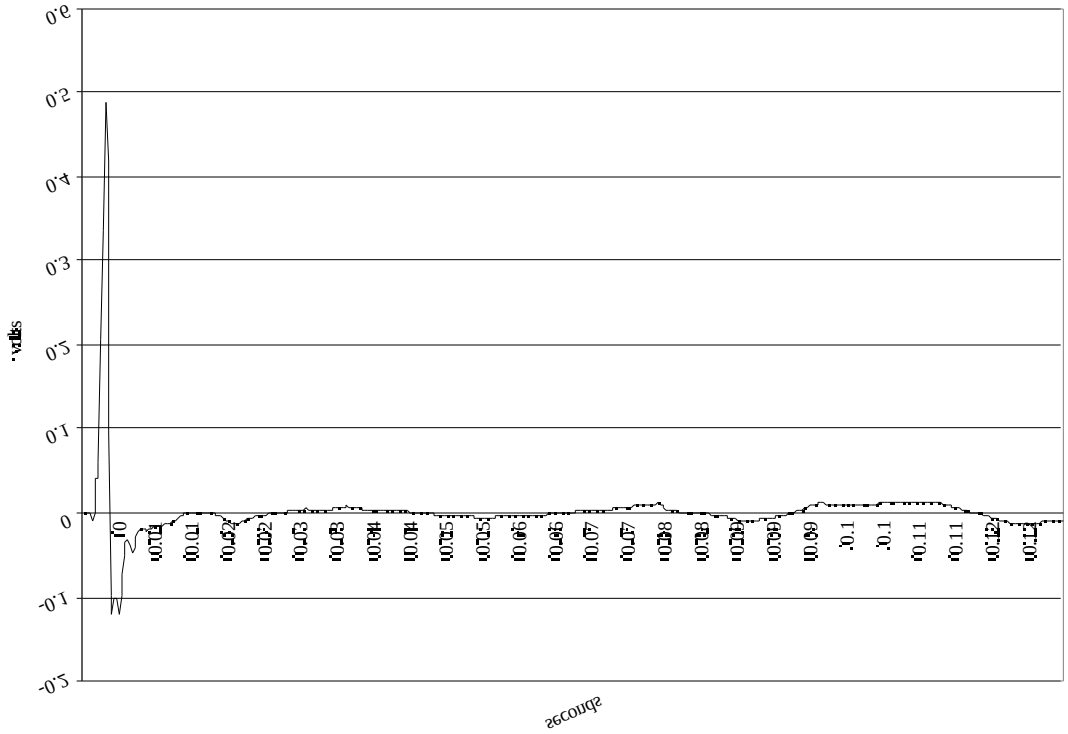
The equipment used to record and analyze the data included a MacLab/8 with Scope v3.5 software, which was wired to feed the data into a MacIntosh computer. The data collected included: animal weight, lidocaine volume, initial recordings of the compound action potentials, the amplitude, onset latency, area under the curve until blockage of evoked potentials is complete, and spread distance of the solution. To obtain the CAP, the nerve was stimulated and the neural response recorded thirty-two times. The average of these thirty-two responses became the CAP used for data analysis. The amplitude was measured as the greatest positive amplitude (mV) after the stimulus. This corresponded to the A-α wave, which is responsible for motor activity. Onset latency was measured as the time from stimulus to the time of first positive deflection in the tracing. Area under the curve was measured from the first positive deflection until the tracing returned to zero volts. The computer calculated the area under this curve. Area under the curve is another test to measure if impulses are transmitted along the nerve. A dye was used to measure how far the solutions travel when injected under the neural sheath. Also, conduction velocity was measured by dividing onset latency by the length of the nerve. Conduction velocity slows as a nerve becomes blocked (Gasser & Erlanger, 1929).

The weight of the Sprague-Dawley rats ranged from 252 grams to 520 grams. There were seven males and three females with the average weight of the animals being 363 grams. Evoked potentials were recorded from these animals before and after being

injected intraneurally with 10 µl of control solution, 20 µl of control solution, 10 µl of 2% lidocaine, or 20 µl of 2% lidocaine. The stimulating voltage for these recordings were 10 times threshold. Threshold was determined to be the minimum amount of voltage needed to create a positive deflection in the recorded neurogram.

Figure 1

Sample Baseline Neurogram at 10 Times Threshold (volts) versus Time (seconds) in the Sciatic Nerve



The recorded neurogram included the Aα, Aβ, Aγ, and Aδ nerve fiber action potentials (Figure 1). The amplitude was recorded as the distance from the 0 volt line to the most positive part of the CAP. This represented the Aα peak which is responsible for muscle contraction. This neurogram contained 32 sweeps, which means it was

stimulated 32 times and the responses were averaged. A biological amplifier was used to increase the recorded response by 100 times.

Table 1

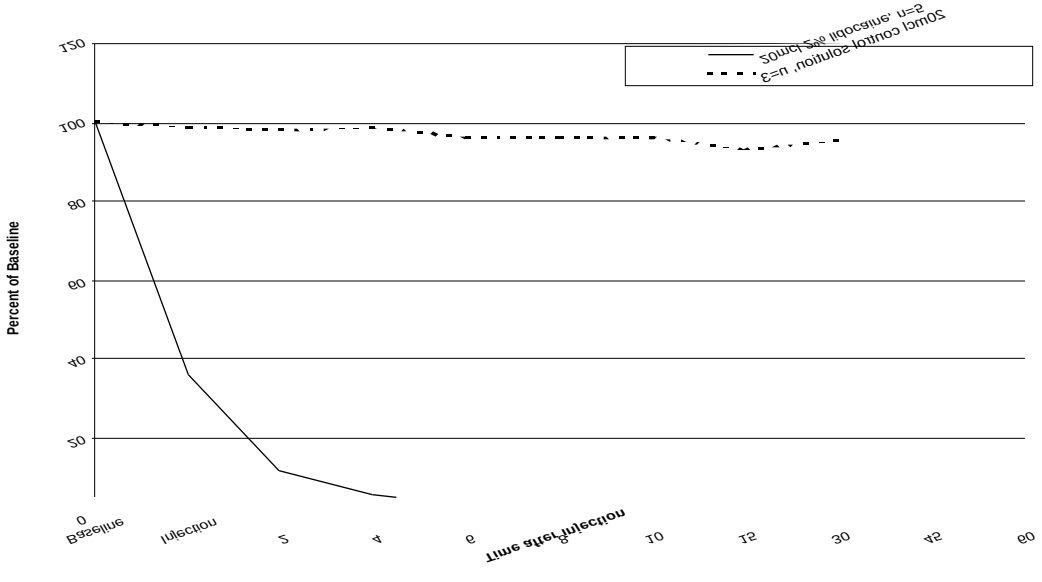
Lidocaine (2%, 20µl) and Control Solution (20µl) effects on Peak Amplitude (mV) versus Time (msec) in the Sciatic Nerve

	Rat #2 lido.	Rat #3 lido.	Rat #5 lido.	Rat #7 lido.	Rat #13 lido.	Rat #15 control	Rat #16 control	Rat #16 control
Base	100	100	100	100	100	100	100	100
Inject	18.359	76.076	26.657	0.498	57.500	99.726	100.12	96.43
2 min	0.103	41.097	0.411	CB	15.969	100.99	97.020	95.441
4 min	0.203	23.201	CB	CB	1.871	101.26	100.37	93.918
6 min	CB	11.665	CB	CB	CB	101.25	96.101	91.743
8 min	CB	5.526	CB	CB	CB	100.69	94.939	92.657
10 min	CB	0.033	CB	CB	CB	98.443	96.752	93.710
15 min	CB	CB	CB	CB	CB	95.102	95.006	89.171
30 min	1.317	CB	CB	CB	CB	99.493	96.752	90.516
45 min	2.115	CB	CB	CB	CB	-	-	-
60 min	-	CB	CB	0.788	CB	-	-	-

Data are reported as percent of pre-injection control values
CB = Complete Block
- = no data

Figure 2

Lidocaine (2%, 20µl) and Control Solution (20µl) effects on Peak Amplitude (mV) versus



Time (msec) in the Sciatic Nerve

Table 1 and Figure 2 show what percent of the baseline of the peak amplitude attained after injection with 20µl of 2% lidocaine or 20µl of control solution. All animals that received 20µl of 2% lidocaine showed a decrease in amplitude until the evoked potentials were completely blocked. The animals that received 20µl control solution showed a slight decrease in amplitude but not less than 89% of baseline. Of note is that this protocol was not amended to add the use of d-Tubocurare until after rat #7. There was no observable twitch for rat #2 at 30 and 45 minutes and there was no observable twitch for rat #7 at 60 minutes. In Table 1, the spaces labeled "CB" in the 2% lidocaine group represent a block of evoked potentials. The spaces with a dash indicate where recordings were not averaged or obtained.

Table 2

Lidocaine (2%, 10µl) and Control Solution (10µl) effects on Peak Amplitude (mV) versus Time (msec) in the Sciatic Nerve

	Rat #8 lido.	Rat #9 lido.	Rat #12 lido.	Rat #15 lido.	Rat #16 lido.	Rat #13 control	Rat #13 control	Rat #15 control
Base	100	100	100	100	100	100	100	100
Inject	98.802	41.247	69.087	58.167	6.2944	90.109	102.43	100.59
2 min	18.744	15.638	39.473	42.666	CB	89.166	95.371	99.435
4 min	0.447	3.084	25.374	28.073	CB	88.760	92.185	98.293
6 min	0.7158	CB	16.416	25.192	CB	87.281	84.125	98.026
8 min	0.3071	CB	11.958	36.392	CB	-	-	97.334
10 min	0.2178	CB	10.555	51.676	CB	88.233	70.793	96.360
15 min	0.0528	CB	10.880	68.959	CB	83.269	69.505	89.238
30 min	CB	CB	13.158	73.075	CB	-	-	88.922
45 min	1.1837	CB	15.239	70.173	CB	-	-	-
60 min	0.9235	CB	18.298	67.820	CB	-	-	-

Data are reported as percent of pre-injection control values
CB = Complete Block
- = no data

Figure 3

Lidocaine (2%, 10µl) and Control Solution (10µl) effects on Peak Amplitude (mV) versus Time (msec) in the Sciatic Nerve

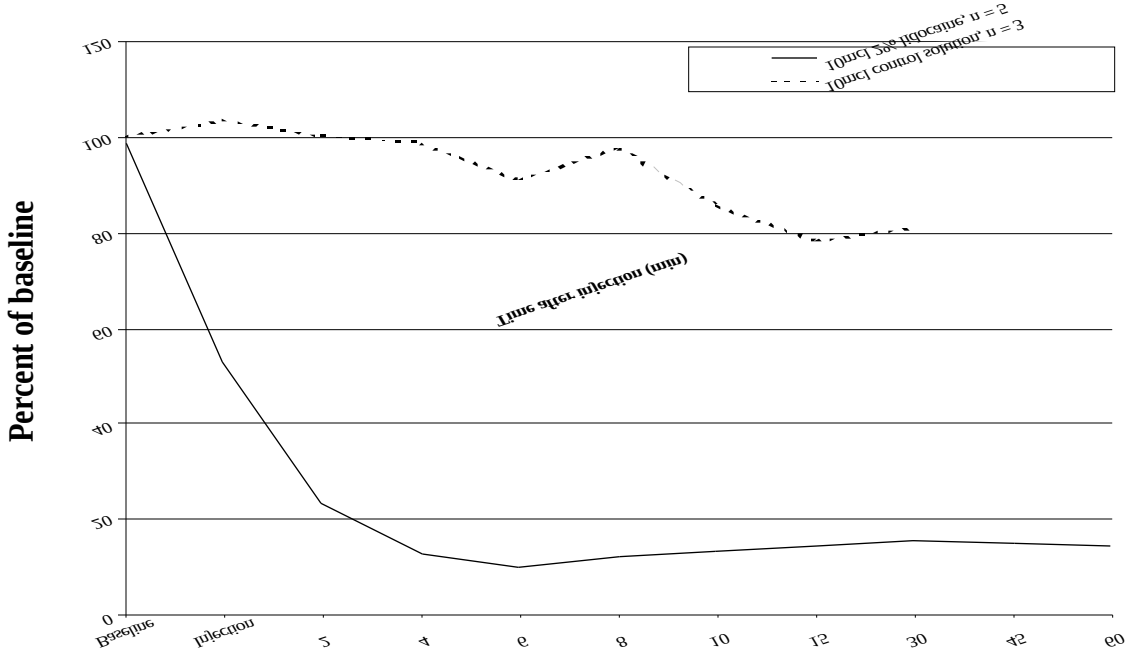


Table 2 and Figure 3 show the percent of baseline that the peak amplitude attained was after injection with 10µl of 2% lidocaine or 10µl of control solution. All animals in this group showed a decrease in amplitude after injection with 2% lidocaine, additionally, 3 of the 5 animals had a complete block of the evoked potentials. The animals that received 10µl of control solution showed a slight decrease in amplitude but not less than 69% of baseline. All animals in this group received d-Tubocurare. In Table 2, the spaces labeled "CB" in the 2% lidocaine group represent a complete block of evoked potentials. The times indicated with a dash, in the control group data are intervals where recordings

were not averaged or obtained.

Table 3

Lidocaine (2%, 20µl) and Control Solution (20µl) effects on Onset Latency (msec) versus Time (msec) in the Sciatic Nerve

	Rat #2 lido.	Rat #3 lido.	Rat #5 lido.	Rat #7 lido.	Rat #13 lido.	Rat #15 control	Rat #16 control	Rat #16 control
Baseline	1	0.75	0.9	0.9	0.6	1	1	1
Injection	1	0.75	1	1.4	0.7	1	1	1
2 min.	2	1	2.1	CB	0.8	1	1	1
4 min.	2	1	CB	CB	0.9	1	1	1
6 min.	CB	1.25	CB	CB	CB	1	1	1
8 min.	CB	1.5	CB	CB	CB	1	1	1
10 min.	CB	3.5	CB	CB	CB	1	1	1
15 min.	CB	CB	CB	CB	CB	1	1	1
30 min.	1	CB	CB	CB	CB	1	1	1
45 min.	1	CB	CB	CB	CB	-	-	-
60 min.	-	CB	CB	1.6	CB	-	-	-

Data are reported in milliseconds
CB = Complete Block
- = no data

Latency of the A-wave CAP was also measured. The onset was measured, in milliseconds, from the end of the stimulus to the point in the neurogram that the tracing of the A-wave CAP started to become positive above the isoelectric line. Table 3 shows the onset time in milliseconds for baseline and after injection with 20µl of 2% lidocaine or 20µl of control solution. The group receiving 20µl of control solution did not show an increase in onset latency. The group receiving 20µl of 2% lidocaine showed a trend towards increase in onset latency. In Table 3, the spaces labeled “CB” in the 2% lidocaine group represent a block of evoked potentials. The times indicated with a dash are intervals where recordings were not averaged or observed

Table 4

Lidocaine (2%, 10µl) and Control Solution (10µl) effects on Onset Latency (msec) versus Time (msec) in the Sciatic Nerve

	Rat #8 lido.	Rat #9 lido.	Rat #12 lido.	Rat #15 lido.	Rat #16 lido.	Rat #13 control	Rat #13 control	Rat #15 control
Baseline	1	1.9	0.8	1	1	0.6	0.7	1
Injection	1	1.9	0.8	1	1.1	0.6	0.6	1
2 min.	1.1	1.9	0.8	1	CB	0.6	0.6	1
4 min.	1.4	1.8	0.8	1	CB	0.6	0.6	1
6 min.	1.3	CB	0.9	1.1	CB	0.6	0.6	1
8 min.	1.4	CB	0.8	1.1	CB	-	-	1
10 min.	1.3	CB	0.8	1.1	CB	0.6	0.6	1
15 min.	1.4	CB	0.9	1.1	CB	0.7	0.6	1
30 min.	CB	CB	0.9	1	CB	-	-	1
45 min.	1.2	CB	0.8	0.9	CB	-	-	-
60 min.	1.1	CB	0.8	0.9	CB	-	-	-

Data are reported in milliseconds
CB = Complete Block
- = no data

Table 4 shows the onset time in milliseconds for baseline and after injection with 10µl of 2% lidocaine or 10µl of control solution. This group did not show a consistent increase in onset latency. Again, two of the animals receiving 10µl of 2% lidocaine and all who received 10µl of control solution did not have a block of evoked potentials. In Table 4, the spaces labeled “CB” in the 2% lidocaine group represent a block of evoked potentials. The times indicated with a dash are where recordings were not averaged or observed.

Table 5

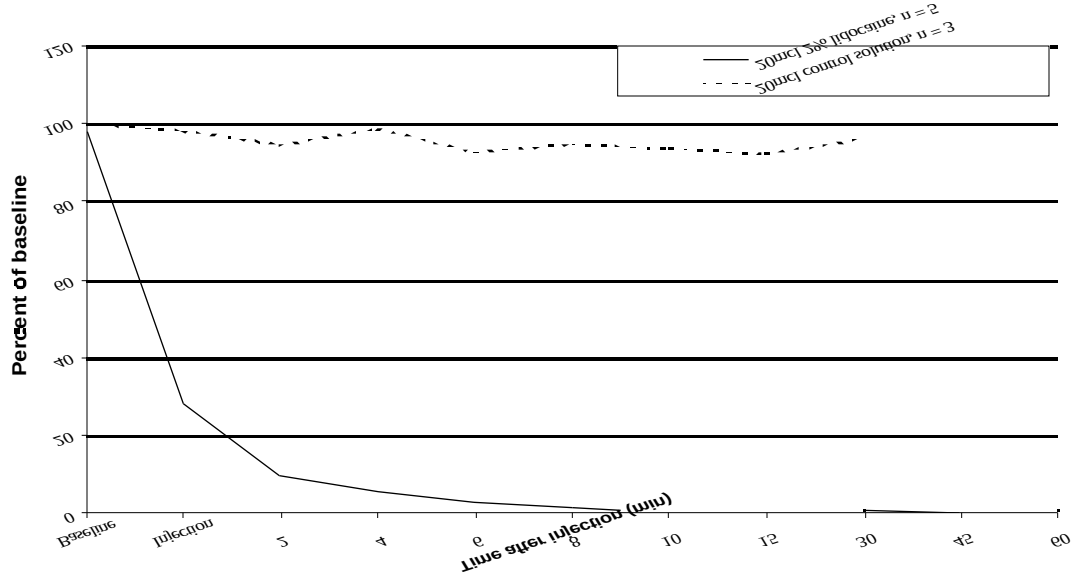
Lidocaine (2%, 20µl) and Control Solution (20µl) effects on Area Under the Curve for Compound Action Potential (V/msec) versus Time (msec) in the Sciatic Nerve

	Rat #2 lido.	Rat #3 lido.	Rat #5 lido.	Rat #7 lido.	Rat #13 lido.	Rat #15 control	Rat #16 control	Rat #16 control
Base	100	100	100	100	100	100	100	100
Inject	21.978	69.543	20.255	3.0534	26.042	98.696	99.065	97
2 min	-0.366	39.086	0.2315	CB	8.3333	99.130	89.720	95
4 min	0.366	23.604	CB	CB	1.0417	100.87	100	95
6 min	CB	10.914	CB	CB	CB	99.565	87.851	91
8 min	CB	4.0609	CB	CB	CB	99.565	89.720	95
10 min	CB	CB	CB	CB	CB	95.652	90.654	95
15 min	CB	CB	CB	CB	CB	96.522	91.589	89
30 min	0.733	CB	CB	CB	CB	100.44	93.458	95
45 min	0.733	CB	CB	CB	CB	-	-	-
60 min	-	CB	CB	0.6794	CB	-	-	-

Data are reported as percent of pre-injection control values
CB = Complete Block
- = no data

Figure 4

Lidocaine (2%, 20µl) and Control Solution (20µl) effects on Area Under the Curve for Compound Action Potential (V/msec) versus Time (msec) in the Sciatic Nerve



The area under the curve was also recorded. The evoked potentials, of all the animals that received 20 µl of 2% lidocaine, were blocked by 10 minutes post-injection as indicated by the area under the curve being equal to 0% when compared to baseline (Table 5 and Figure 4). The evoked potentials were blocked an average of 4.5 minutes with a range of 2 to 10 minutes. In Table 5, the spaces labeled "CB" in the 2% lidocaine group represent a block of evoked potentials. The times indicated with a dash are where recordings were not averaged or observed.

Table 6

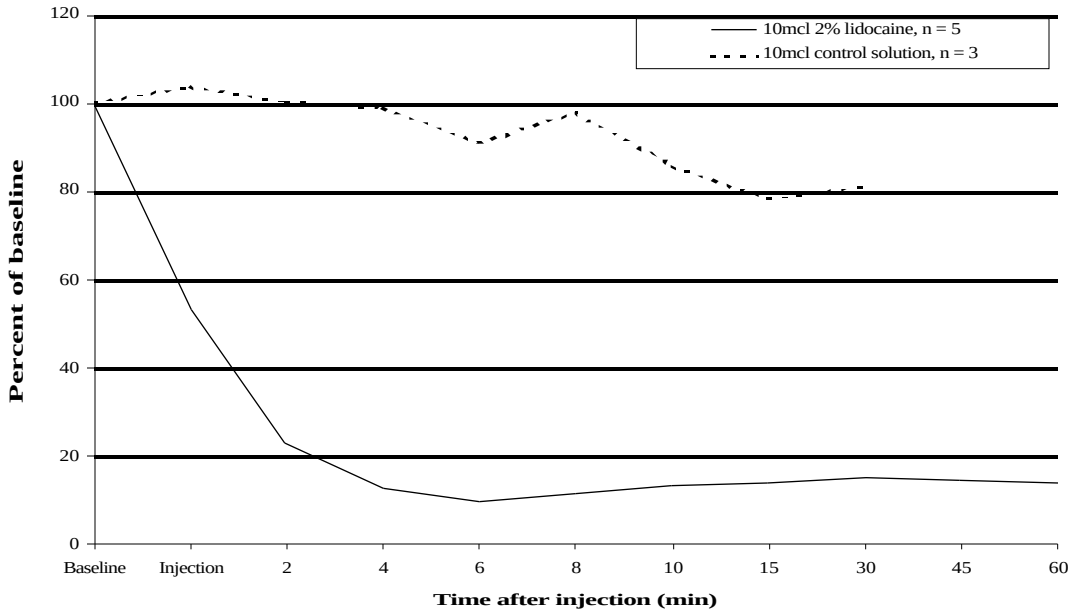
Lidocaine (2%, 10µl) and Control Solution (10µl) effects on Area Under the Curve for Compound Action Potential (V/msec) versus Time (msec) in the Sciatic Nerve

	Rat #8 lido.	Rat #9 lido.	Rat #12 lido.	Rat #15 lido.	Rat #16 lido.	Rat #13 control	Rat #13 control	Rat #15 control
Base	100	100	100	100	100	100	100	100
Inject	103.59	37.017	63.860	50.217	10.588	90.946	123.71	96.273
2 min	17.796	10.055	39.220	47.619	CB	97.787	105.41	97.516
4 min	-3.719	4.1437	27.105	35.065	CB	98.189	100.26	98.137
6 min	-2.112	CB	17.043	33.766	CB	85.714	88.144	99.068
8 min	-1.062	CB	12.526	46.320	CB	-	-	97.826
10 min	-1.049	CB	11.088	56.277	CB	90.946	72.680	93.478
15 min	-0.385	CB	9.4456	60.606	CB	78.068	72.680	83.851
30 min	CB	CB	12.526	62.771	CB	-	-	81.056
45 min	2.656	CB	14.579	55.844	CB	-	-	-
60 min	-0.066	CB	17.659	52.381	CB	-	-	-

Data are reported as percent of pre-injection control values
CB = Complete Block
- = no data

Figure 5

Lidocaine (2%, 10µl) and Control Solution (10µl) effects on Area Under the Curve for Compound Action Potential (V/msec) versus Time (msec) in the Sciatic Nerve



The evoked potentials, of all but two of the animals that received 10 µl of 2% lidocaine, were completely blocked by 30 minutes post-injection as indicated by the area under the curve being equal to 0% when compared to baseline (Table 6 and Figure 5). For the animals that obtained a block, the evoked potentials were blocked by an average of 18 minutes with a range of 2 to 30 minutes. In Table 6, the spaces labeled “CB” in the 2% lidocaine group represent a block of evoked potentials. The times indicated with a dash are where recordings were not averaged or observed.

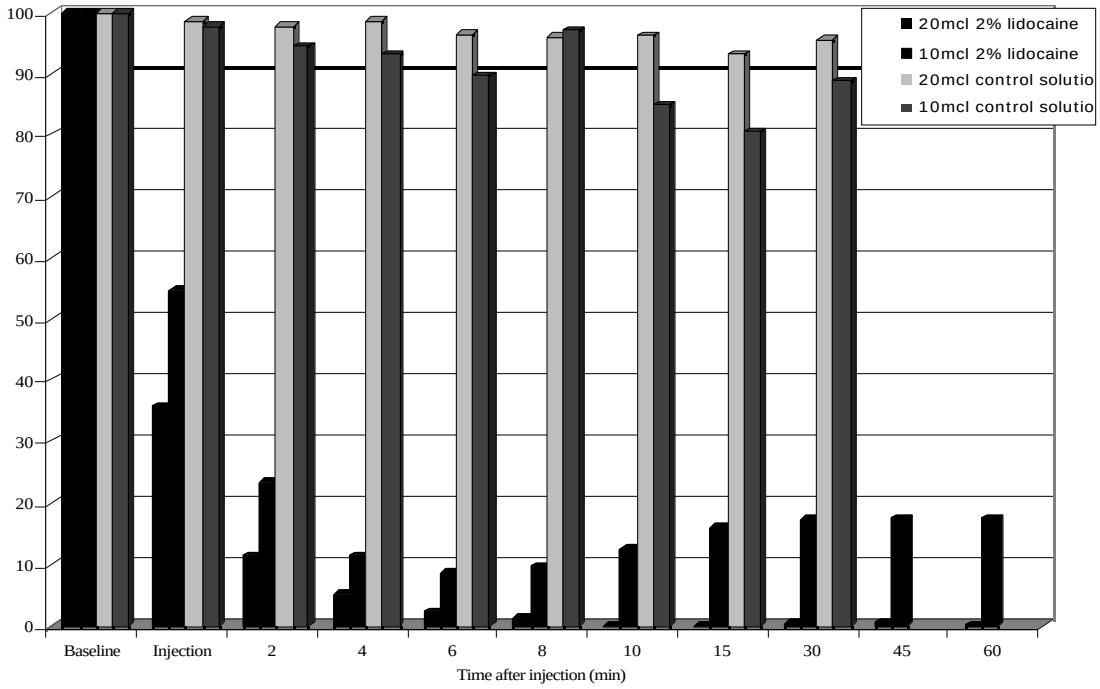
CHAPTER SIX: DISCUSSION

ANOVA and other repeated measures analysis of the data were attempted, but because of sample size, there were insufficient data points to accomplish the analysis. Performing a t-test analysis of the data was not an option due to the increased chance for a type 1 error. For these reasons, the results can only be discussed as trends. The results from these experiments are similar to the results obtained by Paris et al (1990). This study showed a trend for 20μl of 2% lidocaine injected under the neural sheath of the rat sciatic nerve to block the nerve's evoked potentials. This was a strong trend in that all five animals that received the 20μl of 2% lidocaine did have a complete block. The group that received 10 μl of 2 % lidocaine did not show a consistent block of the evoked potentials.

Measurements were done on the A wave CAP which includes Aα, Aβ, Aγ, and Aδ nerve fibers. Due to the short distance between the stimulating and recording electrodes, these A nerve fiber action potentials did not have enough time to separate out and thus measurements were done on the entire compound action potential (CAP). The peak of the compound action potential represents the Aα nerve fibers which are responsible for motor function for the sciatic nerve.

Figure 6

Lidocaine (2%, 10 and 20µl) and Control Solution (10 and 20µl) effects on Peak Amplitude (mV) versus Time (msec) in the Sciatic Nerve



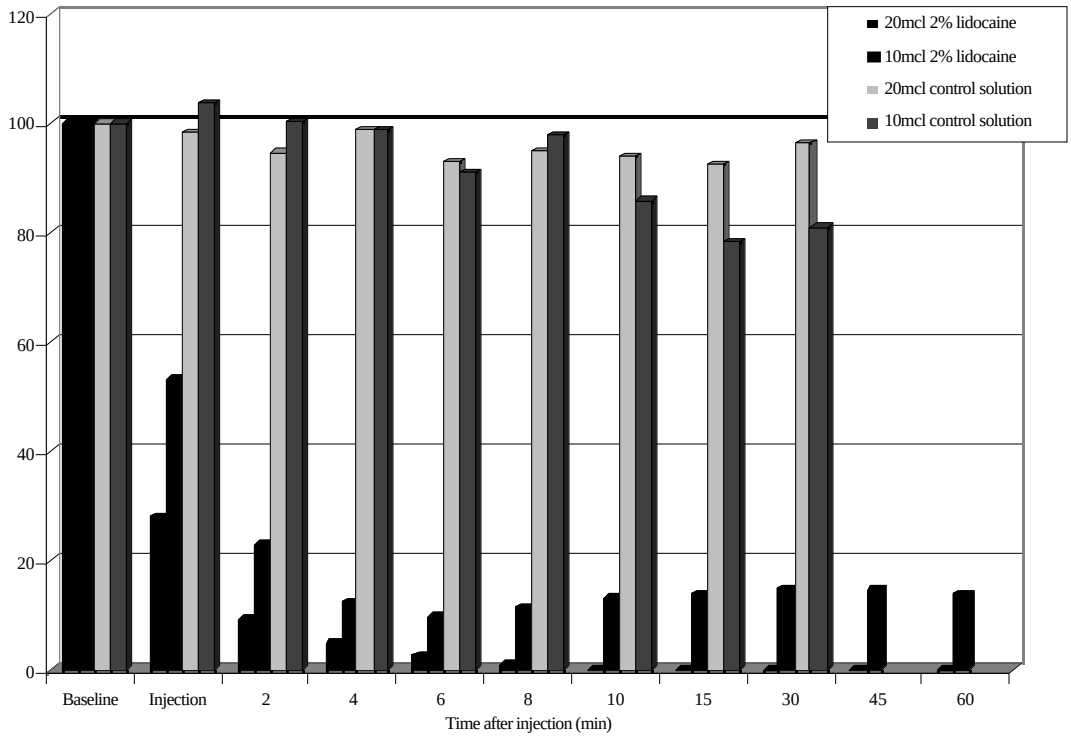
Data are displayed as percent of pre-injection control values

Again, measurements were taken of the peak positive deflection in the compound action potentials and not for individual nerve fiber groups. Sensory (A beta, A gamma, and A delta) activity signals were embedded in the after peak action potential. A reduction to 0% of baseline in the peak amplitude would represent a block of impulse transmission to muscle fibers. The peak amplitudes of the CAPs for all the groups of animals that were tested in shown in Figure 6. It shows that the group that received 20 µl of 2% lidocaine had a reduction to 0% of baseline as soon as 10 minutes post injection.

The group receiving 10 µl of 2% lidocaine on the average did have a reduction but only to about 10% of baseline. The control groups did have a slight reduction in average amplitude. Also, the control groups were not tested at the 45 and 60 minute intervals.

Figure 7

Lidocaine (2%, 10 & 20µl) and Control Solution (10 & 20µl) effects on Area Under the Curve for Compound Action Potential (V/msec) versus Time (msec) in the Sciatic Nerve



Data are displayed as percent of pre-injection control values

Figure 7 compares the area under the curve of the CAPs for all the groups of animals that were tested. It shows that the group that received 20 µl of 2% lidocaine had a reduction to 0% of baseline as soon as 10 minutes. The group receiving 10 µl of 2% lidocaine on the average did have a reduction but only to about 10% of baseline. The

control groups did have a slight reduction in average area under the curve. Also, the control groups were not tested at the 45 and 60 minute intervals.

Figure 8

Lidocaine (2%, 10 & 20µl) and Control Solution (10 & 20µl) effects on Conduction Velocity (m/sec) versus Time (msec) in the Sciatic Nerve

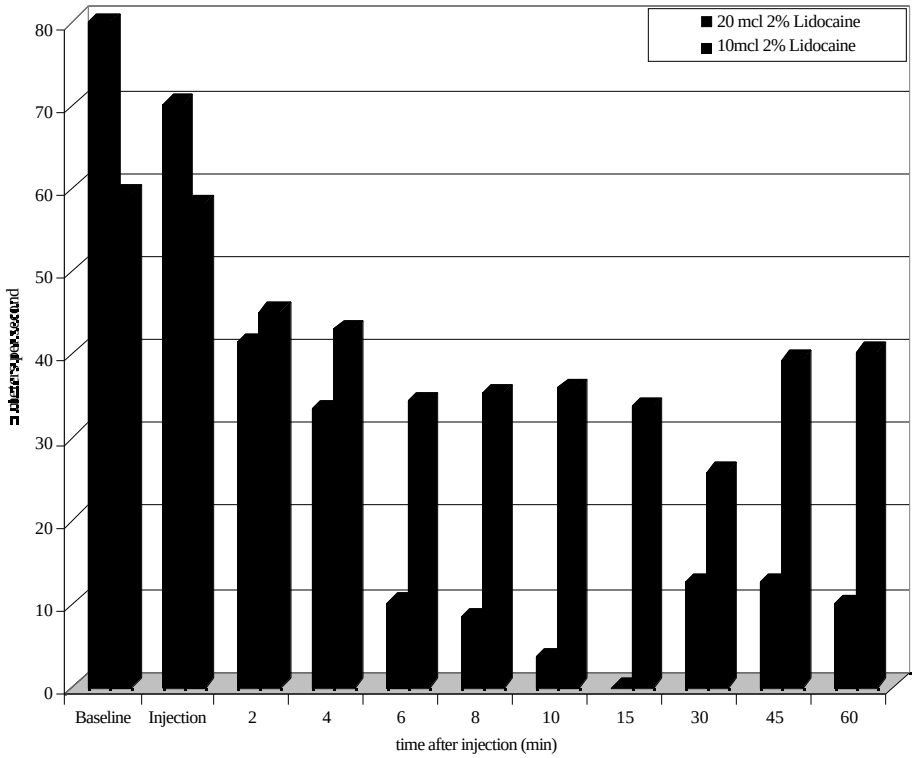


Figure 8 compares the conduction velocity of the CAPs for both experimental animal groups that were tested. Gasser and Erlanger (1929) wrote, "conduction becomes slower before it is extinguished" (p.583). It shows that the group that received 20 µl of 2% lidocaine had an average reduction in conduction velocity to 0 meters/second at 15 min when all the animals had a complete block. The conduction velocities started to

increase in this group when the nerve started to recover from the block. The group receiving 10 µl of 2% lidocaine on the average did have a reduction but only to an average of less than 30 meters/second.

Popitz-Bergez et al. (1995) used radioactive lidocaine to determine the dose found intraneurally after a sciatic nerve block in rats. They found that only 1.6 ± 0.12% of the total dose injected was present intraneurally during complete motor blockade. Using this data, the amount of lidocaine needed to be injected extraneurally to equal 20 µl, the dose needed in this study to reliably block CAPs, would be 1.25 ml of lidocaine. This is 12.5 times the dose that Popitz-Bergez et al. found. This comparison assumes that all 20 µl of lidocaine injected intraneurally was absorbed by the nerve. Also, this study used 2% lidocaine where Popitz-Bergez et al. used 1% lidocaine so it assumes that volume and not concentration effects absorption. In comparing their results to those found by Fink et al., Popitz-Bergez et al. wrote, "Grossly, it appears that intraneural drug content is proportional to injected dose (volume) and not concentration" (p. 590).

In making the correlation between this study and the study done by Popitz-Bergez et al. in 1995, the dose of 12.5 ml of 2% lidocaine is still half of the dose of 25ml of local anesthetic that is recommended by Stoelting and Miller (1994) in their book Basics of Anesthesia.

In conclusion, the animals that received 20 µl of 2% lidocaine had a reduction in the amplitude and the area under the curve to 0% of baseline, and it also increased the onset latency. The animals that received 10 µl of 2% lidocaine also had a reduction in

the amplitude and the area under the curve but not consistently to 0% of baseline, and they also did not have a consistent effect on onset latency.

Recommendations

My recommendations for future studies would be to repeat this experiment with more animals so that the data could be analyzed with a repeated measures test. For these statistical tests to be most effective, I would place each animal in each group, for example one animal would have received injections of 10 µl of control solution, 20 µl of control solution, 10 µl of lidocaine, and 20 µl of lidocaine. I would also recommend using this methodology to test other local anesthetics, different concentrations of local anesthetics, and record data until the CAP return to baseline value.

Another limitation to this study was the amount of time needed. Each experiment took one day to complete. This study had many problems with time constraints in order to complete the number of experiments originally intended. To save valuable time and resources, multiple experiments were done on the later animals but not on the first animals. As stated before it would have been preferable to do the same number of experiments on all animals.

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APPENDICES

APPENDIX A

Protocol Submitted to Laboratory Animal Review Board, USUHS form 6006

USUHS FORM 6006 (LARB) ANIMAL STUDY PROPOSAL FORM

PROTOCOL COVER SHEET

CHECK THE FOLLOWING:

[**X**] New New protocol number: _____ (REA will assign)

[ ] Previously Submitted Old protocol number:

[ ] No modifications

[ ] Minor modifications (indicate all revisions with a ***bold/italic type font***)

PROTOCOL TITLE: The Minimum Effective Dose of Lidocaine Needed to Block Evoked Potentials in the Sciatic Nerve of the (adult Sprague-Dawley) Rat.

PRINCIPAL INVESTIGATOR:

1LT Bradley Stelflug 15 April 1997

Principal Investigator Signature Date

DEPARTMENT: Graduate School of Nursing

TELEPHONE: 295-6565

SCIENTIFIC REVIEW:

/ D.D. Rigamonti PhD, Howard Bryant PhD, Ken Miller PhD /(thesis committee); CAPT Jane McCarthy PhD(Chairman of / department) 15 April 1997

Research Unit Chief/Department Head Signature Date

COORDINATION:

A. <u>Attending/Consulting Veterinarian</u>:

_____

Attending/Consulting Veterinarian Signature Date

(Only required for USDA, Category D or E proposals.)

B. <u>Statistician</u>:

<u>Dr. Ken Miller PhD</u> <u>15 April 1997</u>

Statistician Signature Date

(The PI may certify that the statistical methods are valid.)

USUHS FORM 6006 (LARB)

ANIMAL STUDY PROPOSAL FORM

PROTOCOL TITLE: The Minimum Effective Dose of Lidocaine Needed to Block Evoked Potentials in the Sciatic Nerve of the (adult Sprague-Dawley) Rat.

DATE: 9 April 1997

PRINCIPAL INVESTIGATOR: 1LT Bradley Stelflug

DEPARTMENT: Graduate School of Nursing

CO-INVESTIGATOR(S): Dr. D. Rigamonti, Dr. H. Bryant, Dr. K. Miller

TECHNICIAN(S): N/A

I. NON-TECHNICAL SYNOPSIS: This study will describe the minimum volume of local anesthetic needed to block the conduction of nerve impulses along the sciatic nerve of a Sprague-Dawley rat. The diameter of the animal's sciatic nerve is approximately the size of a human intercostal nerve. Currently, large volumes of local anesthetic are used to block nerve impulses along human nerves which has the potential of systemic toxicity, nerve compression, and tissue necrosis. This study will show what are the minimum volumes (concentration of clinical grade commonly used local anesthetic) needed to block nerve impulses.

II. BACKGROUND:

A. Background: Large volumes of local anesthetic are currently recommended for regional anesthesia of human peripheral nerves. Using these large volumes has the risks of systemic toxicity which can lead to seizures. Also nerve compression or tissue damage from using large volumes can cause nerve damage. Finding the minimum effective volume

could help in reducing the volumes currently being recommended.

B. Literature Search:

1. Literature Source(s) Searched: MEDLINE(R) 1966-1997; AGRICOLA 70, Feb 1997; PsycINFO (R) 1967-1997; EMBASE 1974-1997; BIOSIS PREVIEWS (R) 1969-1997; CAB Abstracts 1972-1996.

2. Date and Number of Search: 11 March 1997

3. Key Words of Search: Sciatic Nerve, Local (Anesthe? Anasthe?, or Anaesthe?), Evoked Potential, Nerve or Neuron, (Vitro or Isolate or Culture), Frog, Minimum Dose, Nembutal, Pentobarbital.

4. Results of Search: The literature showed that this would not be a duplication of effort. The only use of this in vivo model was in 1990 and written in abstract form only published in Abstracts: Society for Neuroscience, Vol.16, 1990, p.183. The model was shown to be a valid model to test local anesthetics and that much smaller volumes of local anesthetics are needed for nerve blockade than are suggested clinically. The literature search shows that the sciatic nerve of a rat can be used to test local anesthetics, that will be used in humans, and that higher phylogenetic species are not necessary for this experiment. The experiments, using frog nerves, used in vitro models and were bathed in solutions which would preclude using an in vitro model for finding the minimum effect volume. Pateromichelakis and Prokopiou (1988) found discrepancies between results of mepivacaine in using in vivo versus in vitro models. Tsirlis et. Al. (1994) found that at a higher pH, lidocaine block had a quicker onset and longer duration of action. This is why an in vivo model using a mammal which would be at physiologic

pH is needed.

III. OBJECTIVE\HYPOTHESIS: To find the minimum effective dose of local anesthetic needed to block nerve transmission in the sciatic nerve of the Sprague-Dawley rat.

IV. MILITARY RELEVANCE: In both the operational theater and in peace time, anesthesia providers will be providing regional blocks to military personnel. Currently, large doses of local anesthetic are being used to perform these blocks. These large doses can lead to nerve damage from compression, tissue death from compression, or even systemic toxicity which can lead to seizures. If smaller doses of local anesthetics were used, then the possibility of these complications would be reduced.

V. MATERIALS AND METHODS:

A. Experimental Design and General Procedures:

1. Experiment # This is a quantitative study to determine the minimum amount of Lidocaine (2%) needed to block recorded evoked potentials in the sciatic nerve of the rat. A total of 18 adult Sprague-Dawley rats are needed. Animals are ordered in quantities of one to two animals per week, which is how many trials are done per week. There will be three animals used to learn the surgical technique of these procedures and then used in the control group. Three additional animals will be in the control group and three animals will be in each of the groups receiving lidocaine.

Number of Animals

6 animals	Group 1	Control group (all volumes)
3 animals	Group 2	Group receiving 10µl of lidocaine
3 animals	Group 3	Group receiving 20µl of lidocaine
3 animals	Group 4	Group receiving 30µl of lidocaine
3 animals	Group 5	Group receiving 40µl of lidocaine
18 animals		**Total**

The techniques will be taught by Dr. Rigamonti, a researcher who has already performed these procedures. The sciatic nerve is tested in vivo in animals initially anesthetized using Nembutal (50 mg/kg ip) as recommended in USUHS "The use of animals in research: A guide for investigators. Supplemental doses will be administered as needed (as tested by corneal reflex). The animals' trachea is surgically exposed and a tracheal tube with T-piece is inserted surgically. A ventilator is available if the animal is in need of artificial respiration. The carotid artery is cannulated in order to draw arterial blood gases to prevent respiratory alkalosis or respiratory acidosis. The left sciatic nerve is surgically exposed and a pair of "stimulating" electrodes are placed on the nerve at the greater sciatic notch and another pair of "recording" electrodes are placed on the nerve around the tibial division near the ankle. The electrodes are embedded in wax in order to avoid possible signal interference from surrounding tissue and also to ensure that the electrical impulse goes only to the nerve. The nerve is tied off proximal to the stimulating electrodes and

distal to the recording electrodes. The nerve is stimulated with a 0.2 ms duration supramaximal pulse every 5 seconds. Ten to fifty responses are averaged. After baseline values are obtained an injection, of lidocaine or control solution, is made inside the nerve sheath and then post injection recordings are obtained. Recordings are made for two hours after injection. The compound action potentials are amplified, visualized, and averaged using MacLab program. Core temperature is physiologically maintained at 37.5^0 +/- 0.3^0 C. An abstract reports that 20µl of lidocaine (2%) is the volume needed to completely block evoked potentials in the sciatic nerve of the rat. The trials are performed at the volumes of 10µl, 20µl, 30µl, and 40µl. The control group (consisting of 6 animals) is injected with a preparation of a rat Ringer solution prepared of $NaHCO_3$ 27.2mM, NaCl 118mM, KCl 4.8mM, KH_2PO_4 1.0mM, $MgSO_4$ 1.2mM, $CaCl_2$ 2.5mM, and glucose 11.1mM to determine how much influence volume plays in blocking evoked potentials. The solution has an osmolarity of 300 +/- 5 mosM and a pH of 7.4 +/- 0.05. The control group is then given a second injection of the rat Ringer solution in which Methylene Blue will be added to measure the spread of the volume in the nerve. No recordings will be made after the injection with Methylene Blue, but the diffusion distance will be measured. All solutions (ringer or lidocaine) are injected into the nerve under microscopic conditions, distal to the stimulating electrodes, using a glass micropipette with a tip OD<30µ placed under the perineurium. The remaining 12 animals (3 per volume group) are injected with Lidocaine 2% which is sold commercially to be used clinically on humans. Data to be collected include: weight of rat, volume of Lidocaine used, initial recordings of the compound action potentials, the amplitude,

latency, area under the curve until blockage of evoked potentials is complete, spread distance of the solution, and length of nerve will be measured to determine conduction velocity. The data collected, which includes the above plus when each fiber group is blocked, is described in a quantitative manner. At the end of the procedure, the animals will be euthanized by giving an overdose of Nembutal (200mg/kg ip) and then their chests will be opened.

B. Laboratory Animals Required and Justification:

1. Non-animal Alternatives Considered: This is an in vivo model which will look at the affects of lidocaine on the sciatic nerve in its homeostatic environment. This will include the whole animal affect. There is no computer model developed for this model.

2. Animal Model(s) and Species Justification: Sprague-Dawley rats were chosen because they were used in the Paris model. To be able to use these results with humans, a warm blooded species was needed. The Sprague-Dawley rat is the smallest animal that still has a nerve that can be compared to a human nerve (The animals sciatic nerve and the human intercostal nerve).

3. Total Number of Animals Required:

Genus & Species Rattus norvegius

Stock/Strain Sprague-Dawley

Sex Either **Age/Weight/Size** adult

Source/Vendor _____

Holding Location(s) Dept.LAM (cental animal facility)

Number of Animals requested/total number of housing days required:

	Animals Requested	**Housing Days**
Year 1	18	126 (18 x 7)
Year 2	N/A	N/A
Year 3	N/A	N/A
Year 4	N/A	N/A
Year 5	N/A	N/A

Special Considerations:

Other: Animals will be ordered such that will only have the number of animals needed for that week.

(Note: This table should be blocked and copied, and repeated for each species requested)

4. Refinement, Reduction, Replacement:

a. Refinement: The procedure will have an early endpoint in that it will end one hour after the evoked potentials are blocked and recovery of nerve function will not be explored.

b. Reduction: Baseline data, of evoked potentials without volumes of solutions added, will be gathered from the animals that will be used for learning the procedure. Since the volume of solution used in the controls will disperse and its effects will be short lived, four volumes will be used with each control animal.

c. Replacement: There are no computer models developed for this in vivo model. Cell cultures will not allow use to test the "whole animal" affect of

this nerve block.

C. Technical Methods:

1. Prolonged Restraint: There will be no prolonged restraint used.

2. Surgery:

a. Procedure: The animals will be anesthetized using Nembutal 50 mg/kg ip. Supplemental doses will be administered as needed (as tested by corneal reflex). The animals' trachea surgically exposed and a tracheal tube with T-piece is surgically inserted. A ventilator is available if the animal is in need of artificial respiration. The carotid artery is cannulated in order to draw arterial blood gases to prevent respiratory alkalosis or respiratory acidosis. The left sciatic nerve will then be surgically exposed at the greater sciatic notch and around the tibial division near the ankle. The nerve will be tied off proximal to the stimulating electrodes and distal to the recording electrodes. A pair of "stimulating" electrodes will be placed on the nerve at the greater sciatic notch and another pair of "recording" electrodes will be placed on the nerve around the tibial division near the ankle. After the animal is euthanized, the sciatic nerve will be dissected to determine the length between electrodes (to be used to determine conduction velocity).

b. Pre- and Postoperative Provisions: The animals will be anesthetized using Nembutal 50 mg/kg ip. The animals will be euthanized at the study endpoint.

c. Location: Building C, room 2081 or 2079

d. Multiple Survival Surgery Procedures:

(l) Procedures: N/A

(2) Scientific Justification: N/A

3. Animal Manipulations:

a. Injections: See section V.C.7.b.

b. Biosamples: Arterial Blood Gases

c. Animal Identification: Date will be placed on the cage.

d. Behavioral Studies: N/A

e. Other procedures: Oscilloscope with MacLab.

f. Location where procedure will take place: Bldg C Rm 2079/81

4. Adjuvants: N/A

5. Study Endpoint: After complete blockage of evoked potentials is accomplished, then stimulations and recordings will be every 5 minutes for one more hour. This will be the study endpoint and the animals will be euthanized because the sciatic nerve will be paralyzed from it being tied off.

6. Euthanasia: The animals will be sacrificed by giving an overdose of Nembutal 200 mg/kg followed by opening the chest to create a pneumothorax. The primary investigator or Dr. Rigamonti will give the overdose of Nembutal.

7. Pain:

a. USDA APHIS Form 7023 Pain Category:

(1) No Pain _____ (#) _____ % (Column C)

(2) Alleviated Pain 18 (#) 100 %

(Column D)

(3) Unalleviated Pain or Distress _____ (#)

_____ % (Column E)

b. Pain Alleviation:

(1) Anesthesia/Analgesia/Tranquilization: The animals will be anesthetized using Nembutal 50 mg/kg ip.

(2) Paralytics: N/A

c. Alternatives to Painful Procedures:

(1) Source(s) Searched: MEDLINE(R) 1966-1997; AGRICOLA 70, Feb 1997; PsycINFO (R) 1967-1997; EMBASE 1974-1997; BIOSIS PREVIEWS (R) 1969-1997; CAB Abstracts 1972-1996.

(2) Date of Search: 11 March 1997

(3) Key Words of Search: Sciatic Nerve, Local (Anesthe? Anasthe?, or Anaesthe?), Evoked Potential, Nerve or Neuron, (Vitro or Isolate or Culture), Frog, Minimum Dose, Nembutal, Pentobarbital.

(4) Results of Search: The literature showed that this would not be a duplication of effort. The only use of this in vivo model was in 1990 and written in abstract form published in Abstracts: Society for Neuroscience, Vol.16, 1990, p.183. The model was shown to be a valid model to test local anesthetics and that much smaller volumes of local anesthetics are needed for nerve blockade than are used clinically. The literature search showed that the sciatic nerve of a rat can be used to test local anesthetics that will be used in humans and that higher phylogenetic species are not

necessary for this experiment. The experiments, using frog nerves, used in vitro models and were bathed in solutions which would preclude using an in vitro model for finding the minimum effect volume. Pateromichelakis and Prokopiou (1988) found discrepancies between results of mepivacaine in using in vivo versus in vitro models. Tsirlis et. Al. (1994) found that at a higher pH, lidocaine block had a quicker onset and longer duration of action. This is why an in vivo model using a mammal at physiologic pH is needed.

d. Painful Procedure Justification: The animals will be anesthetized using Nembutal 50 mg/kg ip to avoid causing any pain to the animals from the procedure. The animals will be euthanized in a rapid and painless way by giving Nembutal 200 mg/kg ip at the study endpoint before the initial anesthesia wears off.

D. Veterinary Care:

1. Husbandry Considerations: The animals will be cared for at UHUHS Central Animal Facility until they are used for the study. The animals will be ordered so that the number arriving each week will be used that week (usually 1 animals per week, may be up to 2). The total number of housing days was figured by each animal staying a maximum of 7 days.

a. Study Room: N/A

b. Special Husbandry Provisions: None needed.

2. Attending Veterinary Care: Animals that are in need of Veterinary care will be seen by a Veterinarian from the department of Laboratory Animal Medicine. The investigator will follow any recommendations made by the Veterinarian for the animal.

3. Enrichment Strategy:

a. Dogs: N/A

b. Nonhuman Primates: N/A

E. Data Analysis: Data will be analyzed with the MacLab statistical software package. This is a standard statistical package for analyzing evoked potentials. It has the capabilities to record and average the responses, measure the amplitude and latency and area under the curve until blockage of evoked potentials is complete.

F. Investigator and Technician Qualifications/Training: Brad Stelflug, Don Rigamonti, and Howard Bryant will be performing all the procedures in the entire experiment. Dr. Rigamonti will be present for all the procedures. He helped develop the model that will be used and he has done these procedures before. Dr. Rigamonti and Brad Stelflug attended the USUHS Rodent Handling Course on 30 April 1997. Dr. Bryant has worked with rodents before on protocols at USUHS. Dr. Rigamonti, Dr. Bryant, and Brad Stelflug have attended an approved USUHS Investigator Training Course.

VI. BIOHAZARD/SAFETY:

VII. ASSURANCES: As the Primary Investigator on this protocol I provide the following assurances

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a deviation is specifically approved by the LARB.

B. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with an individual who is qualified to evaluate the statistical design or strategy of this proposal, and that the "minimum number of animals needed for scientific validity are used."

D. Biohazard/Safety: I have taken into consideration, and I have made the proper coordinations regarding all applicable rules and regulations regarding radiation protection, biosafety, recombinant issues, etc., in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures/manipulations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused as a result of the procedures/manipulations.

F. Training: I verify that I have attended an approved Uniformed Services University of the Health Sciences (USUHS) Investigator Training Course.

G. Training: The following personnel will attend the next approved USUHS Investigator Training Course:

1LT Brad Stelflug (Done 04 March 1997)

Dr. D. Rigamonti (Done 04 March 1997)

Dr. H. Bryant (completed)

H. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to

conduct this study in the spirit of the fourth "R" which the DoD has embraced, namely, "Responsibility" for implementing animal use alternatives where feasible, and conducting humane and lawful research.

_____

Principal Investigator Signature Date

I. Painful Procedures: (Include above if conducting research that will cause more than slight or momentary pain or distress [Column D or E by USDA classification] the following statement must follow.) **I am conducting biomedical experiments which may potentially cause more than momentary or slight pain or distress to animals that WILL BE relieved or WILL NOT (circle one) be relieved with the use of anesthetics, analgesics and/or tranquilizers.** I have considered alternatives to such procedures; however, using the methods and sources described in the protocol, I have determined that alternative procedures are not available to accomplish the objectives of the proposed experiment.

_____

Principal Investigator Signature Date

VIII. ENCLOSURES:

A. Literature Searches: MEDLINE(R) 1966-1997; AGRICOLA 70, Feb 1997; PsycINFO (R) 1967-1997; EMBASE 1974-1997; BIOSIS PREVIEWS (R) 1969-1997; CAB Abstracts 1972-1996.

B. Pathology Addendum:

C. Pain Scoring Guidelines:

D. <u>Adjuvant Policy</u>:

IX. PROTOCOL ABSTRACT:

A. Protocol Number: (if new, leave blank) _____

B. Title: The Minimum Effective Dose of Lidocaine Needed to Block Evoked Potentials in the Sciatic Nerve of the (adult Sprague-Dawley) Rat.

C. Principal Investigator: 1LT Bradley Stelflug

D. Performing Organization: Uniformed Services of the Health Sciences

E. Funding: from the Graduate School of Nursing

F. Objective and Approach: This study will describe the minimum volume of local anesthetic needed to block the conduction of nerve impulses along the sciatic nerve of a Sprague-Dawley rat. The diameter of the animal's sciatic nerve is approximately the size of a human intercostal nerve. Currently, large volumes of local anesthetic are used to block nerve impulses along human nerves which has the potential of systemic toxicity, nerve compression, and tissue necrosis. This study will show what are the minimum volumes needed.

G. Indexing Terms (Descriptors): sciatic nerve, local anesthetic, lidocaine, animals, Sprague-Dawley rats.

APPENDIX B

Biohazards, Controlled Substances And Dangerous Materials,

USUHS Form 6007 (BCD)

USUHS FORM 6007 (BCD) BIOHAZARDS, CONTROLLED SUBSTANCES AND DANGEROUS MATERIALS

REA Date Stamp

Protocol No.: **TO6133-01**

Principal Investigator: **Bradley Stelflug**

Department: **GSN** **Phone:** (301) 295-6565

Project Title: **The Minimum Effective Dose of Lidocaine Needed to Block Evoked Potentials in the Sciatic Nerve of the Rat.**

PLEASE NOTE: Failure to provide **ALL** required information could result in project disapproval, or, at least add significantly to the length of the approval process. If you require assistance in preparing this form, contact (as needed) the Environmental Health and Occupational Safety (EHS) Department (295-3321), the Pharmacy Officer (295-3305), or the Pharmacy Technician (295-3668).

BCD categories which apply to this project:

___	Biohazards (Section A)	___	Recombinant DNA {rDNA} (Section B)
X	Controlled Substances (Section C)	X	Chemicals (Section D)
___	Others (Section E)		

SECTION (A) BIOHAZARDS

1. Information to assist in the completion of this section can be found in USUHS Instruction 6401 *"Biohazards and Dangerous Materials Guide,"* and the CDC/NIH Guidebook *"Biosafety in Microbiological and Biochemical Laboratories."*

a. Type of Biohazard Agent to be used:

___	Bacteria	___	Virus	___	Parasite
	Mold/Fungi		Human Blood/Blood Products/Unfixed Tissue		

Others _____

b. Specific Biohazard Agent(s) to be used:

Biohazard Agent	Required Biosafety Level	Room(s) Location(s)	Immunizations Available (Yes/No)

c. I**mmunoprophylaxis:** The use of safe and effective vaccines approved by the FDA for at-risk personnel is required where available. Less efficacious, or partially approved vaccines may be made available. Please list your at-risk personnel (and those support people who may become at-risk) and state whether they are willing to receive vaccination, or, sign a waiver.

Name(s) of affected staff	Vaccination Y/N	Waiver Y/N

d. Human Blood or Blood Products or Unfixed Tissue: If human blood, blood products, bodily fluids and/or secretions, or unfixed tissues will be used in this project, complete the following:

Name(s) of affected staff	Hepatitis B Vaccination Y/N	Blood-Borne Pathogen Training in past 12 mo. (Date of last training)

e. Handling of Biohazards or Human Blood/Blood Products/Unfixed Tissue:

Describe in sufficient detail to permit evaluation of how the agent(s) will be handled. Account for source, storage, experimental procedures, and disposal methodology. Emphasize precautions to be taken to protect laboratory personnel and the public.

SECTION (B) RECOMBINANT DNA (rDNA)

2. Guidelines for the use of recombinant DNA can be found in the *USUHS Guidelines for Research Involving Recombinant DNA Molecules*. A copy is available in EHS and additional copies can be made available to requesting departments.

a. Have you read the USUHS rDNA Guidelines? ☐ Yes ☐ No

b. Please provide the following information:

Source(s) of DNA/rDNA: _____

Nature of inserted DNA sequences _____

Host(s) and Vector(s) to be used: _____

Will expression of a foreign gene be attempted? ___ Yes ___ No

If yes, what protein(s) will be produced? _____

According to the "Guidelines", what is the proposed Class of study?

_____

What level of containment will be used (Section II, "Guidelines")? _____

If used, what biological barriers are incorporated? _____

SECTION (C) CONTROLLED SUBSTANCES

3. Have you reviewed USUHS Instruction 6404 "Management of Controlled Substances, Alcohol and Alcohol Liquors, Prescription Medications, Hypodermic Needles, Syringes, List I and II Chemicals, and Anabolic Steroids"?

a. Controlled Substances List: List all controlled substances to be used. List by generic form (no brand names), provide concentrations of substances obtained as premixed medications (i.e. 100 mg/ml), and indicate the laboratory, room or other location where the substance(s) will be used**. List only controlled substances and anabolic steroids.** Do not re-list these materials in Section D.

Generic Name	Amount/Concentration	Location
Sodium Pentobarbital	50 mg/ml	C2081/79

b. Safeguards: Describe in sufficient detail to permit evaluation, how the controlled substance(s) will be obtained (source), secured, used, accounted for, ***and what method(s) will be employed for disposal of unused quantities and for waste materials generated for their use.*** Identify safeguards and precautions to eliminate possible loss or abuse. **REMINDER: Return all "unused" quantities of controlled substances and anabolic steroids to the Pharmacy.**
Sodium Pentobarbital (50 mg/ml) purchased from the USUHS pharmacy will be given to the physiology department's drug custodian (Howard Bryant Ph.D.) to be stored in the safe in room C2081. The amount of Sodium Pentobarbital (50 mg/ml) required for a day's experiments will be obtained from the drug custodian. Any unused drug will be returned to the drug custodian at the end of that day.

c. List I and List II Precursor/Essential Chemicals: The following chemicals, their slats, isomers, and slats of optical isomers in threshold amounts are List I and List II Precursor/Essential

Chemicals and are subject to Federal requirements under the Chemical Diversion and Trafficking Act of 1988. Please provide the information requested on those List I and List II Precursor/Essential Chemicals you will use. Do not re-list these chemicals in Section D; however, all training and other requirements of Section D apply here as well.

Acetone	Acetic Anhydride	Anthranilic Acid
Benzyl Cyanide	Benzyl Chloride	Ethyl Alcohol (95%)
	Ephedrine	
Ethyl Alcohol (Abolute)	Ergonovine	Ergotamine
Hydriotic Acid	N-acetylanthranilic acid	Norpseudoephedrine
Phenylacetic acid	Phenylpropanolamine	Piperidine
Potassium permangenate	Pseudoephedrine	2-butanone
Methyl Ethyl Ketone	Toluene	

3,4-methylenediodeoxyphenyl-2-propanone

Table of List I and List II precursor/essential chemicals with their intended use and location

Chemical Name	Intended Use (i.e., solvent)	Location	Hazard Class	Handling, Storage & Personal Protection	Waste Disposal
Ethyl Alcohol	Solvent	C2081/79	F	FC,GL	2

Codes to be used in above table

Hazard Class	Storage	Handling & Personal Protection	Waste Disposal
F=Flammable P=Poison CR=Corrosive CC=Carcinogen E=Explosive I=Irritant M=Mutagen T=Teratogen H=Highly Toxic N=Non-Toxic D=Drug O=Other	FC=Flammable Cabinet SC=Storage Cabinet AC=Acid Cabinet BC=Base Cabinet SS=Special Cabinet ES=Explosive Storage OS=Strong Oxidizing Agent	LC=Lab Coat GL=Gloves EG=Eye Goggles FS=Face Shied SM=Surgical Mask RP=Respirator EP=Ear Protector FH=Fume Hood BS=Biosafety Cabinet	**1**- Stored in a glass container sealed & labeled as hazardous waste & removed by EHS for disposal **2**- Highly dilute and/or neutralized solution (non-EPA/ RCRA waste); dispose of down the drain. **3**- When disposal of the solid material is necessary, it will be disposed of as solid hazardous waste through EHS.

			waste through EHS.

SECTION (D) CHEMICALS

4. **Guidelines for the proper use of chemicals are published as the *OSHA Laboratory Standard*.** A copy of these guidelines are available in EHS. EHS staff are available for interpretation or amplification of the guidelines.

a. **Have you read:**

OSHA Laboratory Standard?	[ ]	Yes	[X]	No
USUHS Safety Manual?	[X]	Yes	[ ]	No
USUHS Waste Disposal Guide?	[X]	Yes	[ ]	No
USUHS Chemical Hygiene Plan?	[X]	Yes	[ ]	No

Is your laboratory in compliance with the requirements of the *OSHA Laboratory Standard* and *USUHS Chemical Hygiene Plan*?

[X] Yes [ ] No

Have you considered the following during design of this protocol?

1. **The environmental impact of use of the chemicals involved in your study?**

[X] Yes [ ] No

2. **The use of less hazardous, or less toxic, chemicals?**

[X] Yes [ ] No

3. **Ordering only the amount of chemicals you can utilize in your study in order to minimize chemical waste due to outdated or unused chemicals?**

[X] Yes [ ] No

4. **Have you established a local common storage site for chemicals?**

[ ] Yes [X] No

b. **Training:** List the current status of those personnel (**including the PI**) who will be working with chemicals (in any form or amount) on this protocol with regard to the **annual** laboratory safety training required by OSHA regulations. If training was provided by other than EHS, provide appropriate documentation.

Name(s) of affected staff	Date of last attendance
Brad Stelflug	5/5/97
Howard Bryant Ph.D.	2/18/97, 4/10/97
Don Rigamonti	

c. **Chemical List: A complete list of *all* chemicals used in this research project must be provided.** Your project cannot be approved if chemicals mentioned in the ***Materials and Methods***, or other sections of your protocol are not addressed on USUHS Form 6007. Standard chemical reagents for each department or laboratory can be documented in a list provided to EHS. If you have such a list on file then you may attach a copy of it to this form **provided you place a check mark (✓) next to the chemicals to be used in this project.** New or additional chemicals should be listed in the table provided below.

CHEMICAL LIST - Chemicals Not Included on a Departmental/Laboratory List

Chemical Name	Hazard Class	Handling, Storage & Personal Protection	Waste Disposal
Lidocaine	D	SS, GL	2

CODES to be used in above list

Hazard Class	Storage	Handling & Personal Protection	Waste Disposal
F=Flammable P=Poison CR=Corrosive CC=Carcinogen E=Explosive I=Irritant M=Mutagen T=Teratogen H=Highly Toxic N=Non-Toxic D=Drug O=Other	FC=Flammable Cabinet SC=Storage Cabinet AC=Acid Cabinet BC=Base Cabinet SS=Special Cabinet ES=Explosive Storage OS=Strong Oxidizing Agent	LC=Lab Coat GL=Gloves EG=Eye Goggles FS=Face Shied SM=Surgical Mask RP=Respirator EP=Ear Protector FH=Fume Hood BS=Biosafety Cabinet	**1**- Stored in a glass container sealed & labeled as hazardous waste & removed by EHS for disposal **2**- Highly dilute and/or neutralized solution (non-EPA/ RCRA waste); dispose of down the drain. **3**- When disposal of the solid material is necessary, it will be disposed of as solid hazardous waste through EHS.

SECTION (E) <u>OTHERS</u>

5. **List other hazards which may be involved in this project** (i.e., UV light, compressed gasses, high intensity lights, lasers, etc.)
N/A

6. **List special safety programs or procedures involved in this project** (i.e., pre-exposure serology, immunization program, physical examination, pulmonary function test, hearing tests, etc.).
N/A

PHARMACY:

7. **Have you consulted with the Pharmacy on your chemical and controlled substance requirements?**

X Yes ___ No

8. **Have you established, with the Pharmacy, a listing of chemicals required by this protocol detaining concentration, grade, size of container, manufacturer, etc.?**

X Yes ___ No

SECTION (G) STATEMENT OF RESPONSIBILITY

STATEMENT OF RESPONSIBILITY:

I have read, and understand, the *USUHS Safety Manual*, the *USUHS Waste Disposal Guide*, and the most recent guidelines for those sections of this form which apply to my proposal. I have been properly trainied and I will see that required training is provided for my staff, and that all who work under this protocol abide by the provisions of the *USUHS Biohazard, Controlled Substances, and Dangerous Materials Program*. I understand that EHS is available for consultation in the training of staff and for other occupational health support when required. I will report to EHS any instances where the safety of personnel or the environment are threatened.

	05 May 1997
Principal Investigator (signature)	**Date**

APPENDIX C

Laboratory Animal Review Board Response Letter

28 May 1997

MEMORANDUM FOR DR. BRADLEY W. STELFLUG, GRADUATE SCHOOL OF NURSING

SUBJECT: Laboratory Animal Care and Use Review of Protocol Number TO6133- 01, "The Minimum Effective Dose of Lidocaine Needed to Block Evoked Potentials in the Sciatic Nerve of the Rat"

Animal Welfare Act, Public Health Service Policy, and USUHS instruction 3204 require the review of all research and teaching protocols involving the use of animals. The USUHS Laboratory Animal Review Board (LARB) has reviewed your Protocol No. TO6133-01, entitled, "The Minimum Effective Dose of Lidocaine Needed to Block Evoked Potentials in the Sciatic Nerve of the Rat." Issues raised in the review of USUHS Form 6006, "USUHS Animal Study Proposal", which require further clarification or explanation including the following:

1. Section II.B.1. All DoD funded research projects are required to conduct a Defense Technical Information search. Please provide evidence that the searches have been completed.

2. Section V.A. Reference is made to drawing arterial blood gases to prevent respiratory alkalosis or respiratory acidosis, but there is no reference made to the frequency or volume of blood withdrawn. Please clarify.

3. Section V.A. What is the volume of rat Ringer solution as control given to the animals? Reference is made that the control group is used to "determine how much influence volume plays", but the volume is not identified.

4. Section V.A. How was the sample size determined? Will three animals per group provide adequate number for statistically significant results?

5. Section V.A. If 20 µl of lidocaine (2%) was reported to block evoked potentials completely, and the aim of the study is to determine the smallest dose required, why is there only one test point (10µl) that is a lower dose? If 20 µl does provide a complete block, then the 30µl and 40 µl volumes should not beused. Instead, doses of 5

µl and 2.5 µl should be used in addition to 10 µl. If volumes smaller than 10 µl present technical difficulties, dilution of the 2% lidocaine should be considered. Please comment.

Respectfully request that you respond to the above by memorandum. You are responsible for sending this memorandum to Research Administration. You are also responsible for sending copies of this memorandum to me (LTC Nathaniel Powell, LAM), Dr. Richard Andre, Preventive Medicine/Biometrics, Dr. Franziska Grieder, Microbiology, Dr. Sara Contente, Pathology, Dr. Gary Francis, Pediatrics, Dr. David Dobbins, Physiology, Dr. Sharon Juliano, Anatomy And Cell Biology, Dr. Cheryl Dicarlo, LAM, Mr. Tim Allen (in care of LAM), Dr. Carol Bossone, Medicine, Ms. Karen Dern, University Affairs, Ms. Olga D'Onofrio (in care of LAM) (LARB members). Your response memorandum will subsequently become part of Protocol No. TO6133-01. Please respond within 60 days of the date on this memorandum. A lack of response within this time frame will necessitate a resubmission of USUHS Form 6006.

Nathaniel Powell, Jr., D.V.M.
LTC, VC, USA
Executive-Secretary, Laboratory Animal
Review Board

Cc:
File
LARB distribution

APPENDIX D

Response Memorandum

21 JULY 1997

MEMORANDIUM FOR LTC NATHANIEL POWELL, D.V.M.
SUBJECT: Protocol No. T06133-01 response memorandum

1. The "Multi-Databases" from the Defense Technical Information Center was searched on 2 July 1997. The query text used were "local anesthetics AND in vivo model" and "local anesthetics AND minimum dose". Two studies were in the general area of my proposed study; "Development of Ultra Long Duration Local Anesthetic Agents in a Rat Model" and "Analysis of the Ability of DMSO (Dimethyl Sulfoxide) and Lidocaine to Penetrate Dentin". These studies are not similar to the proposed study in that they do not test the minimum dose of Lidocaine needed in an in vivo model.

2. After the rat is anesthetized, one of the carotid arteries in the neck will be cannulated for blood gas measurements. Five or less, 0.15 ml samples will be drawn during the course of the experiment and run on a Instrumentation Labs Model 1306 blood gas machine that determines pH, PCO2, PO2, bicarbonate, and hemoglobin.

3. **Control Group Receiving Rat Ringer Solution**

Animal #1	10µl and then 30µl*
Animal #2	20µl and then 40µl*
Animal #3	10µl and then 30µl*
Animal #4	20µl and then 40µl*
Animal #5	10µl and then 30µl*
Animal #6	20µl and then 40µl*

* The second volume will be injected after the evoked potentials have returned to baseline. This will be done to reduce the number of animals used in this study.

4. Sample size was based on data from previous studies. Additionally, the use of repeated measures (specifically for the control group) will provide sufficient data points for achieving statistically significant results. And finally, descriptive studies do not require a power analysis because they are not testing hypotheses but rather describing the data as they exist.

5. The first experimental group will receive the 20µl volume to reconfirm the dose that was published. If 20µl blocks the evoked potentials, then the next experimental group will receive 5µl less (15µl) and so on until the minimum effective dose is reached.

If 20µl does not block the evoked potentials, then the next experimental group will receive 5µl more (25µl) and so on until the minimum effective dose is reached.

1LT Brad Stelflug

APPENDIX E

Laboratory Animal Review Board Approval Letter

27 October 1997

MEMORANDUM FOR DR. BRADLEY W. STELFLUG, GRADUATE SCHOOL OF NURSING
SUBJECT: LARB Approval of Protocol

The following application was reviewed and approved by the Uniformed Services University of the Health Sciences (USUHS) Laboratory Animal Review Board (LARB) on 3 October 1997:

Title of Application: "The Minimum Effective Dose of Lidocaine Needed to Block Evoked Potentials in the Sciatic Nerve of the Rat"

Protocol Number: TO6133-01

Name of Principle Investigator: Dr. Bradley Stelflug

The USUHS has a Animal Welfare Assurance on file with the Office for Protection from Research Risks, National Institutes of Health. The Assurance Number is A3448-01. The LARB approved the above referenced application as submitted.

Nathaniel Powell, Jr., D.V.M.
LTC, VC, USA
Executive-Secretary, Laboratory Animal Review Board

cc:
Research Administration

APPENDIX F

Protocol Modification

27 February 1998

MEMORANDUM FOR EXECUTIVE SECRETARY, LABORATORY ANIMAL REVIEW BOARD (LARB)

SUBJECT: Request for Minor Modifications to Protocol #TO6133-01, entitled, "The Minimum Effective Dose of Lidocaine Needed to Block Evoked Potentials in the Sciatic Nerve of the Rat"

The objective of these studies is to determine the minimal dose of local anesthetic (lidocaine) needed to block the sciatic nerve in a rat. To date, we have successfully recorded the electrical activity of the nerve before and after anesthetic injection. We have also been able to visually note the loss of motor activity in the innervated leg muscles after anesthetic injection. However, we are concerned that motor activity, both electrical (EMG) and mechanical (movement artifact) may be contaminating the recorded electrical neural response. Since both of these processes are synchronized with the neural signal, electronic or computer averaging techniques will not eliminate these possible artifacts.

We therefore request modification of our protocol to include the administration of the nondepolarizing neuromuscular blocking agent (d-tubocurare, 0.04-0.06 mg/kg, IV) to the anesthetized animal. This drug should have little or no effect on neural excitability while eliminating the electrical and mechanical response of the muscle. Prior to administration of the blocking agent, the animal will be mechanically ventilated (Volume = 1 cc per 100g of rat weight. 60 - 80 breaths per minute). The drug will be delivered through an indwelling internal jugular catheter containing heparin/saline (heparin 25u/cc) solution. The total time from administrating the muscle relaxant to termination of experiment is approximately two hours. During this time, periodic IP injections (5mg per 30 min. Nembutal) of anesthetic will be administered at intervals, that from our previous experience without muscle relaxant, will maintain a surgical plane of anesthesia. Also we will monitor the animals heart rate by ECG monitoring and supplement more anesthetic for signs of light anesthetic plane (tachycardia).

Bradley W. Stelflug, BSN
1LT, AN, USA

cc:

Dr. Don Rigamonti, Ph.D.
Dr. Howard Bryant, Ph.D.

APPENDIX G

Protocol Modification Temporary Approval Letter

March 2, 1998

MEMORANDUM FOR RECORD

SUBJECT: Minor Modification for Dr. Bradley Stelflug, Graduate School of Nursing, Protocol Number TO6133-01, entitled, "The Minimum Effective Dose of Lidocaine Needed to Block Evoked Potentials in the Sciatic Nerve of the Rat"

On March 2, 1998, Dr. Brad Stelflug, Graduate School of Nursing, requested a minor modification for Protocol number TO6133-01, entitled, "The Minimum Effective Dose of Lidocaine Needed to Block Evoked Potentials in the Sciatic Nerve of the Rat."

Dr. Stelflug's request to include the administration of nondepolarizing neuromuscular blocking agent, D-Tubocurare, to anesthetized rats in the above protocol, is approved. This approval is considered temporary and will be validated at the next full meeting of the USUHS LARB.

Nathaniel Powell, Jr., D.V.M.
LTC, VC, USA
Executive-Secretary, Laboratory Animal
Review Board

cc:
File
Dr. Stelflug
Research Administration

APPENDIX H

Protocol Modification Approval Letter

24 March 1998

MEMORANDUM FOR DR. BRADLEY W. STELFLUG, GRADUATE SCHOOL OF NURSING

SUBJECT: LARB Approval of Minor Modification of Protocol

The following minor modification was reviewed and approved by the Uniformed Services University of the Health Sciences (USUHS) Laboratory Animal Review Board (LARB) on March 18, 1998:

Title of Application: "The Minimum Effective Dose of Lidocaine Needed to Block Evoked Potentials in the Sciatic Nerve of the Rat"

Protocol Number: TO6133

Name of Principle Investigator: Dr. Brad Stelflug

The USUHS has a Animal Welfare Assurance on file with the Office for Protection from Research Risks, National Institutes of Health. The Assurance Number is A3448-01. The LARB approved the above referenced application as submitted.

Nathaniel Powell, Jr., D.V.M.
LTC, VC, USA
Executive-Secretary, Laboratory Animal Review Board

cc:
File
Research Administration